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SEROLOGICAL TESTS WITH STANLEY'S CRYSTALLINE TOBACCO-MOSAIC PROTEIN[1]

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The crystalline protein recently reported by Stanley (6) to possess the properties of tobacco-mosaic virus has been made available to the writer for serological tests. The precipitin, complement-fixation, and anaphylaxis techniques have been used in experiments recently completed. It is the purpose of this paper to present the results of these studies.

MATERIALS AND METHODS

Crystalline Tobacco-mosaic Protein. In the tests reported, with the exception of a few that were preliminary, one or another of 5 samples of crystalline tobacco-mosaic protein was employed. The two samples used in most of the experiments were designated as "Crystals I" and "Crystals II," respectively. Both were of protein that had been recrystallized several times by Dr. Stanley. "Crystals I" was not recrystallized during the course of this work, but "Crystals II" was recrystallized twice before it was tested serologically. The details of treatment of the two samples are as follows.

"Crystals I," which was received in November, 1935, was a suspension of protein at a concentration of approximately 1 per cent in ammonium sulphate solution. The suspension was dialyzed against Ringer salt solution for 20 hours in order to remove the ammonium sulphate and to obtain an approximately 1 per cent solution of the protein in Ringer solution. The protein was then precipitated with .5 saturated ammonium sulphate. The precipitate was taken up in a small volume of Ringer salt solution and dialyzed against this solution until the ammonium sulphate had been removed. The resulting solution contained about 2 per cent of protein. The sample designated as "Crystals II" was received in January, 1936. It was in the form of a paste consisting of protein crystals suspended in ammonium sulphate solution. The protein was recrystallized twice under the direction of H. S. Loring. The second crystallization yielded a suspen-

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sion of crystals that appeared to be free from other materials when examined microscopically. A 2 per cent solution from these crystals was made by suspending an excess of protein in Ringer solution and stirring for 20 hours. This was dialyzed against Ringer solution. The samples designated as "Crystals III, IV, and V" were received in February, 1936. They were submitted as arbitrarily numbered unknowns, and were tested serologically before their identity became known to the writer. After the tests were completed, the information was provided that "Crystals III" was a sample of tobacco-mosaic crystals from mosaic-diseased tobacco, "Crystals IV" was a sample of tobacco-mosaic crystals from mosaic-diseased tomato, and "Crystals V" was similar to "Crystals III," but in a higher state of purification. These 3 samples had been dialyzed against salt solution before they were received, and they were used for serological tests without additional treatment.

Partially-purified Concentrates. Virus in crude frozen juice from tobacco plants having mosaic disease was concentrated by precipitating once or twice with .4 or .5 saturated ammonium sulphate, suspending the precipitate in a small volume of saline solution, and dialyzing against Ringer solution. Three such concentrates were used in these experiments. Several concentrates of the juices of healthy tobacco, mosaic-diseased tomato, and healthy tomato were similarly prepared and used in the tests. The concentrates usually contained about 5 mg. per cc. of protein. Infection tests were used to give assurance that the healthy-plant concentrates contained no virus and that the mosaic-diseased plant concentrates contained abundant virus.

Crude Tobacco Juices. Juices of virus-containing and virus-free tobacco plants were used with no treatment other than freezing, centrifuging, and dialyzing against saline solution.

Other Crude Plant Juices. For purposes of orientation, the anaphylaxis technique was first applied to a variety of viruses and hosts. In these tests crude juices were used. They were prepared in the same manner as the crude tobacco juices. Juices containing the viruses of the following diseases were employed: several strains of tobacco mosaic, tobacco ring spot, peach yellows, peach rosette, little peach, rugose mosaic of potato, mild mosaic of potato, and latent mosaic of potato. The hosts used were potato and tobacco for the potato viruses, peach for the peach viruses, tobacco for tobacco ring-spot virus, and tobacco, petunia, pepper, tomato, and phlox for the tobacco-mosaic viruses.

Precipitin and Complement-fixation Tests. Each of these tests was conducted according to the techniques previously described by the writer (2).

Anaphylaxis Tests. The anaphylaxis test in serology is based on the thesis that an animal injected with a small amount of protein develops,

after about 3 weeks, a high state of sensitivity toward the particular protein used in the sensitizing inoculation. A second inoculation of the same protein after this period causes violent contractions of smooth muscle, frequently resulting in death within a few minutes. The reaction is highly specific. The Schultz-Dale modification of this principle is based on the fact that if a virgin female guinea pig is properly sensitized with a protein, its excised uterus horns, when placed in an isotonic bath, will exhibit contraction when the specific protein used in sensitization is added to the bath. Such contraction may be conveniently recorded on a kymograph drum. The Schultz-Dale modification is somewhat more sensitive than the test in which the animal is reinoculated with protein. It also has the advantage of making possible several tests with the same animal. In the work reported here, the method was used in the manner described by Dale (3), with the exception of certain minor modifications, which will be briefly described.

Since the plant antigens to be studied are normally used in the form of expressed juice, and since the concentration of protein in this juice is relatively low (*e.g.*, healthy-tobacco juice was found to contain only about .2 mg. of protein per cc.), the addition of 1 or 2 cc. of expressed plant juice to the 100-cc. bath usually used may sometimes result in an excessively high dilution of protein. Accordingly, in some of the experiments a bath was used that contained 20 cc. of fluid and permitted the addition of 2 cc. of plant juice, which gave only a 1:10 dilution factor beyond the degree of dilution of the protein in the juice itself. When using such a small bath, it was found desirable to have the plant juices at the same temperature as the bath before adding them, and to have the juices isotonic with the Ringer solution used in the bath. The customary 100-cc. bath was used in comparison with the smaller bath in a number of tests. The comparison showed that the size of the bath did not alter the character of the reactions. The sensitivity of the apparatus was tested by means of experiments in which egg albumin was the antigen used. Reactions were obtained at dilutions consistent with the findings of other workers who have used this technique.

It became apparent early that many plant juices are exceedingly toxic to the excised guinea pig uterus, causing it to contract when the juices are at high dilution. All of the juices tested proved to be so highly toxic that they could not be used without some treatment. Dialysis in a Kunitz-Simms dialyzing apparatus (4) was found to be effective in removing the toxic material from the plant extracts. By dialyzing against Ringer solution it was also possible to replace the unsuitable electrolytes of the juices by the isotonic salt composition of Ringer's solution. Such dialysis usually was continued for 16 to 24 hours, but the toxic material of the potato passed so slowly through the dialyzing membrane that 36–48 hours of dialysis were necessary. The dialyzed extracts could be used freely at a dilution

of 1 : 10 in the bath without the production of artefact contractions. In the case of anaphylaxis, the muscle tissue shows only one complete reaction toward a given antigen; in the case of the toxic materials in plant juices, the tissues react completely several times in succession, provided they are washed and allowed to relax after each contraction. Thus the two types of reaction may be distinguished.

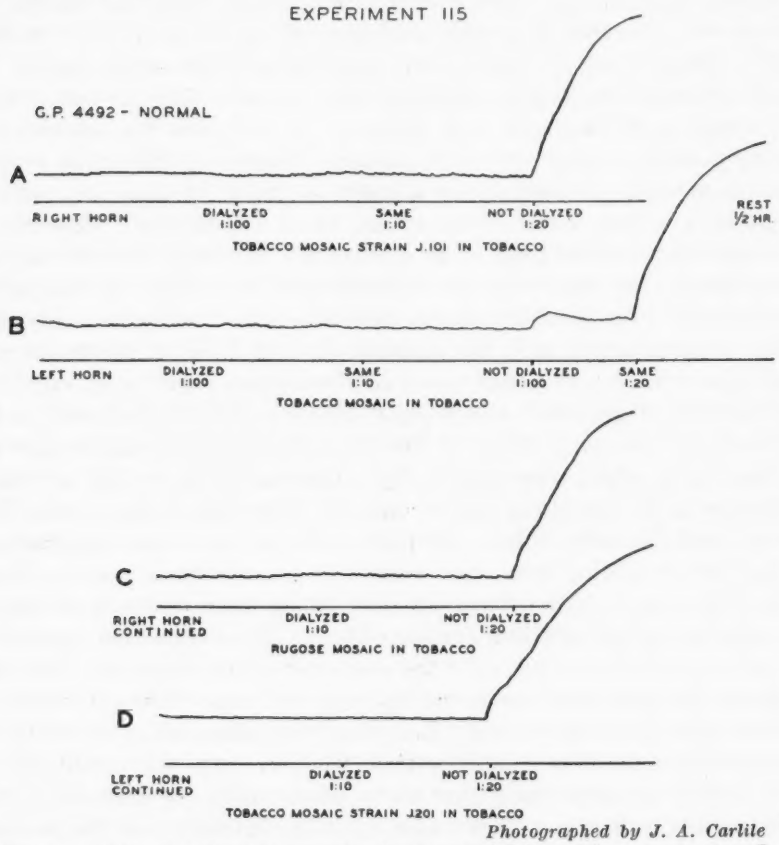

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FIG. 1. Effect of dialysis in removing toxic material from tobacco extracts. In each case the dialyzed extract is nontoxic (*i.e.*, induces no muscle contraction), while the corresponding undialyzed extract is highly toxic.

The efficiency of dialysis in eliminating artefact toxic reactions of plant juices is illustrated in figure 1, which gives the kymograph records of the two horns of a normal guinea-pig uterus treated successively with dialyzed and nondialyzed aliquots of virus-containing tobacco juice. Record A shows that the addition of dialyzed tobacco juice at a net concentration of either 1 : 100 or 1 : 10 failed to cause contraction or even to interrupt the normal

pulsation of the muscle, while the nondialyzed aliquot of the same juice at 1:20 caused violent contraction. After allowing this muscle to relax, it was similarly tested with dialyzed and nondialyzed aliquots of a second tobacco juice sample, with analogous results (Fig. 1, B). The other horn gave similar results with two other tobacco juice samples (Fig. 1, C and D). In the anaphylactic tests reported in this paper, only extracts were used that failed to stimulate the excised muscle of a normal guinea pig in at least two such control tests. Some of the control tests are shown in the figures.

EXPERIMENTAL

1. *Preliminary Anaphylaxis Tests.* The precipitin and complement-fixation tests have been used by a number of workers in the study of plant viruses. By means of these tests, specific reactions for virus materials are obtained. The writer has submitted evidence (1) indicating that it is the virus itself that is the specific antigen involved. The precipitin and complement-fixation reactions with tobacco-mosaic virus are about 25 times as strong as the reactions with the proteins of healthy-tobacco juice. It was learned from preliminary anaphylaxis tests, however, that the healthy-tobacco proteins are highly active anaphylactically. In the purification of tobacco-mosaic virus, the contaminants most difficult to remove are the non-virus proteins of the tobacco plant. This fact suggested the use of the highly sensitive anaphylaxis reaction in the serological study of the crystalline tobacco-mosaic protein; but, before proceeding with this study, orientative tests employing crude and partially-purified virus materials were made.

Previous work with the precipitin and complement-fixation tests had brought evidence that crude plant-virus juice contains two kinds of antigens, namely, proteins normally present in the healthy plant and the specific virus antigen. It is necessary to eliminate the reactions due to healthy-plant proteins, which are present in virus juice, in order to demonstrate reactions due to the virus antigen. For anaphylaxis tests, three ways of eliminating normal host protein reactions suggested themselves, and all were attempted. The three methods were: (a) to permit a sensitized muscle to react fully to healthy-plant juice, and then determine whether there is a residual reaction peculiar to the virus-containing material; (b) to use in sensitizing and testing, respectively, two hosts containing the same virus but so widely separated taxonomically that the only common serological factor would be the virus antigen; and (c) to work with virus materials independently proved to have been freed by chemical means from normal host proteins. The three methods will be discussed in turn.

It is known from the works of numerous investigators that if a guinea pig is sensitized simultaneously to two different proteins, then the excised muscle, when permitted to react completely to an addition of one of these

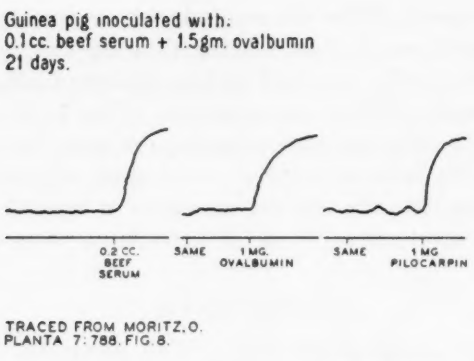

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FIG. 2. Absorption anaphylaxis reaction with beef serum and ovalbumin. Showing that a guinea pig sensitized with a mixture of these two proteins reacts completely to each in succession.

proteins and subsequently allowed to relax, will show a second complete reaction on the addition of the second protein. Figure 2, which was taken from a record of Moritz (5, Fig. 8, p. 788) illustrates this principle. The guinea pig in question was sensitized with a mixture of beef serum protein and egg albumin. When, after a proper incubation period, the excised muscle was tested against beef serum, it reacted strongly. A second dose of beef serum failed to elicit a reaction, indicating that the muscle had been completely desensitized with respect to that antigen, *i.e.*, its anti-beef-serum anaphylactic antibodies were saturated or exhausted. On the addition of egg albumin, however, a second strong contraction occurred. The first reaction with beef serum did not inhibit the occurrence of a second reaction to a distinct protein. A further dosage of egg albumin failed to cause further reaction, indicating that the antibodies for both proteins were exhausted, although the muscle was still physiologically active, as was shown by the fact that the addition of a small amount of pilocarpin, a nonspecific muscle stimulant, caused a third strong reaction. The principle exemplified in this record was applied to virus materials in the following manner.

Tobacco-mosaic virus was chosen for the first anaphylactic tests. The procedure was to sensitize a guinea pig with 1 or 2 cc. of normal-strength or concentrated juice of mosaic-diseased tobacco plants, and then to test successively against healthy-tobacco juice and virus-containing tobacco juice. The results of 3 experiments of this type are given in figure 3.

The records bring out the following points. Guinea pigs that were sensitized to virus-containing tobacco juice reacted to antigens present in healthy-tobacco juice. This indicates that healthy-tobacco juice and virus-containing tobacco juice contain common antigenic materials. The addition of a second aliquot of healthy-tobacco juice failed to induce contraction,

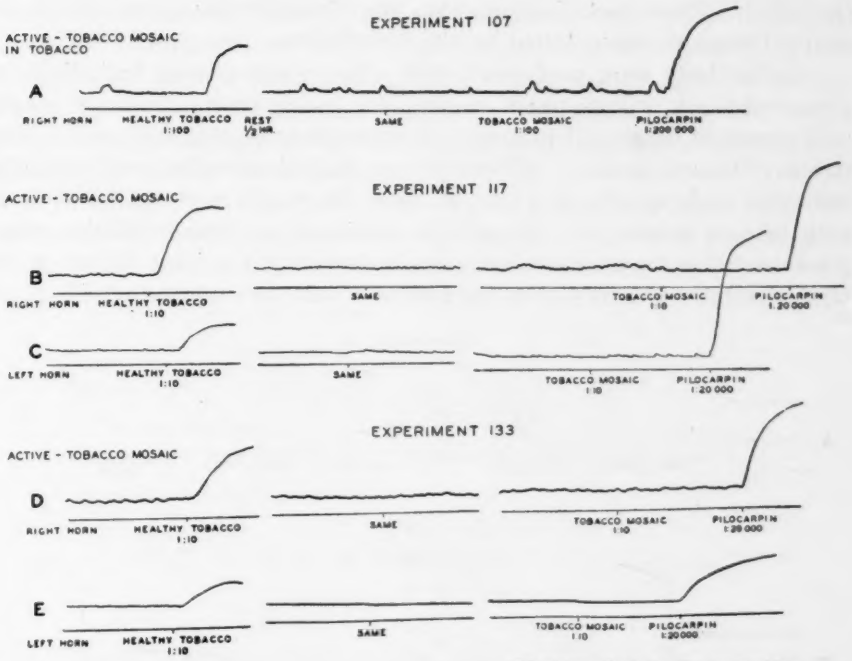

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FIG. 3. Absorption anaphylaxis tests with tobacco-mosaic materials. In each case the animal was sensitized to whole extract of tobacco mosaic in tobacco; in each case the muscle reacted to healthy-tobacco protein, but failed later to react to the virus element of the juice, although the muscle was still active as shown by the reaction to the muscle toxin pilocarpin.

showing that the muscles were now saturated with respect to the healthy-tobacco antigens. On adding samples of virus-containing tobacco juice, no further reactions occurred, although in each case the muscle was still in a state of high physiological activity, as was shown by its strong reaction to the muscle stimulant pilocarpin when the latter was at a concentration not greater than 1:20,000 to 1:200,000. Independent tests with normal guinea pigs showed that the healthy-tobacco and diseased-tobacco extracts used contained no detectable nonspecific toxic material at the dilutions employed.

The 3 experiments reported are representative of others performed with tobacco materials and gave comparable results. The materials used for sensitization and the test materials contained appreciable quantities of virus. In some cases the virus in the test material was at 1000 times the concentration necessary to give a precipitin reaction. We know from precipitin tests that the inoculum contained two classes of antigens, virus antigen and healthy-tobacco antigens, yet the animals reacted only toward

the latter. These facts indicate that the virus of tobacco mosaic is not anaphylactogenic when tested by the Schultz-Dale technique.

Similar tests were performed with other plant viruses including the viruses of peach yellows, peach rosette, little peach, rugose mosaic of potato, mild mosaic of potato, latent mosaic of potato, tobacco ring spot, and several strains of tobacco mosaic. All except the peach viruses give good precipitin tests with their specific sera. In all cases the results were similar to those with tobacco mosaic, *i.e.*, the animals sensitized to virus-containing plant juice reacted to the normal plant protein element of the juice but not to the virus element. Figure 4 gives the results of such an experiment with peach

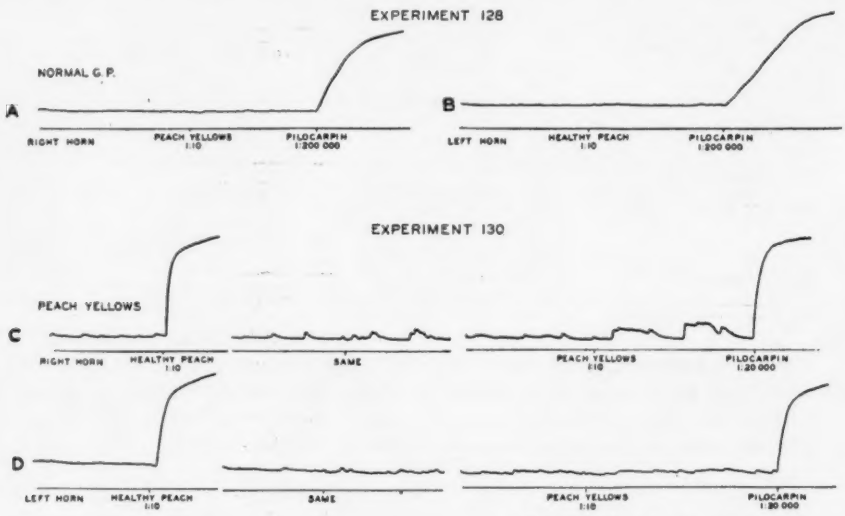

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FIG. 4. Absorption anaphylaxis tests with peach-yellows virus materials. Records A and B show that the dialyzed extracts used were nontoxic to normal guinea pig muscles; records C and D as in figure 3, indicating reactivity to the plant-protein element of the extracts but not to the virus element.

yellows. Records A and B show that the two extracts that were intended for use later in the anaphylactic tests did not stimulate the muscles of a noninoculated guinea pig, *i.e.*, were nontoxic at 1:10 dilution, although the normal guinea-pig muscles were highly sensitive to toxic materials, as shown by their reaction to pilocarpin at 1:200,000. Records C and D are of right and left uterus horns, respectively, of a guinea pig sensitized with 2.5 cc. of dialyzed expressed juice from a yellows tree. The two horns were tested in the same manner. Each reacted fully with juice of healthy peach, but after saturation failed to react with yellows juice, although the pilocarpin reactions showed a high degree of sensitivity of the muscles.

The experiments with tobacco-mosaic virus, peach-yellows virus, and the

other viruses mentioned give evidence that none of these viruses is anaphylactogenic. It was felt, however, that further tests were desirable, since it might be that with the plant materials a first reaction against healthy-plant juice in some way inhibits a second reaction against a virus that would otherwise be anaphylactogenic. In view of this possibility, the following type of experimentation was resorted to.

A guinea pig was inoculated with 3 cc. of a potent preparation of the juice of phlox plants infected with tobacco mosaic. After 21 days of incubation the muscles were tested, with results as shown in figure 5.

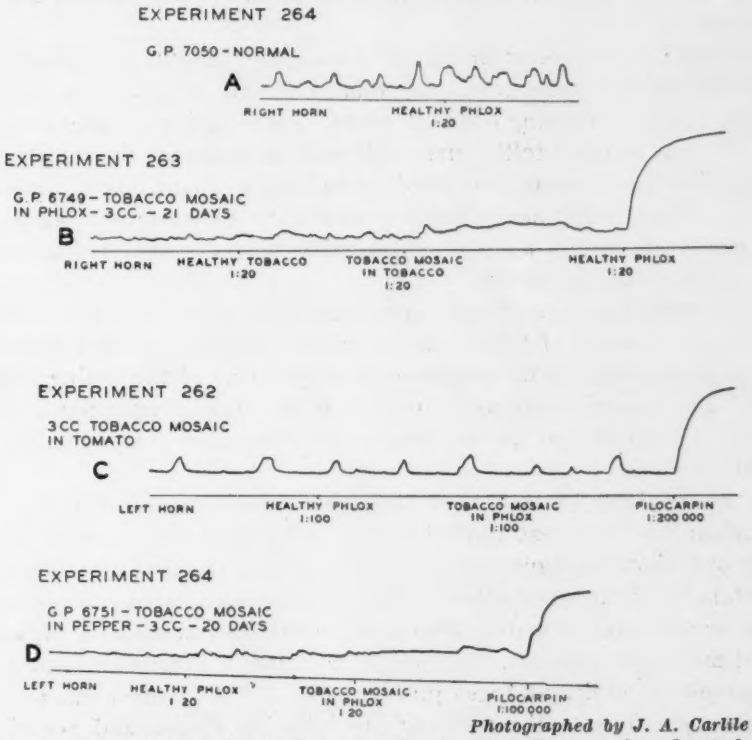

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FIG. 5. Anaphylaxis tests of tobacco-mosaic virus excluding reactions due to healthy-host proteins by appropriate choice of hosts. Record A, a control test of toxicity of phlox extract. Record B shows that a guinea pig sensitized with tobacco mosaic in phlox fails to react both with healthy-tobacco extract and with virus-containing tobacco extract. Records C and D indicate that other host plants gave similar results.

The uterine muscle was first tested against an extract of healthy-tobacco juice (Fig. 5, record B). There was no reaction, indicating that phlox and tobacco are too remotely related to contain common antigenic material. Next, the muscle was tested against an extract of virus-containing tobacco juice and again there was no reaction. Since the muscle had not previously

reacted against anything, the failure of virus reaction could not be attributed to inhibiton by a previous reaction. Furthermore, there was at least one common antigenic factor in the virus-containing phlox and tobacco juices, namely, the virus. Absence of reaction hence indicates, in conformity with the preceding experiments, that the virus of tobacco mosaic is not anaphylactogenic. That the muscle in question was capable of giving an anaphylactic test is shown by the fact that it later reacted strongly to the juice of healthy phlox, and that this was a true anaphylactic reaction was brought out by the control test (Record A), which showed that the same phlox extract at the same dilution failed to stimulate the muscle of a normal guinea pig.

Records C and D show similar tests using as materials for sensitization tomato and pepper juices containing tobacco-mosaic virus, and as test material phlox juice containing the same virus. In no case was there a reaction that might be ascribed to the virus, although in each case the materials for sensitization and testing contained considerable quantities of infectious matter. These experiments are representative of numerous others that gave analogous results and brought further evidence that tobacco-mosaic virus is not anaphylactogenic.

As already has been stated, precipitin tests afford a highly sensitive index of the presence of tobacco-mosaic virus, but they exhibit a rather low order of sensitivity for the presence of the proteins of the healthy tobacco plant. The data given above indicate that the anaphylactic test is highly sensitive as applied to healthy-tobacco proteins and entirely insensitive toward the virus.

2. *Anaphylaxis Tests of the Crystalline Tobacco-mosaic Protein.* The anaphylaxis test was next applied to the crystalline tobacco-mosaic protein. The records shown in figures 6 and 7 are of tests performed with the sample "Crystals I." The crystalline-protein solution was tested against several normal guinea pigs at a net dilution of 1:800, and in no case caused the normal muscle to contract. Record B in figure 6 illustrates such a test. Two samples of healthy-tobacco juice were used, "A", which was prepared 3 months previous to the tests by precipitating frozen and centrifuged healthy-tobacco juice with 40 per cent saturated ammonium sulphate and by taking up the precipitate in 1/35 the original volume of diluent, and "B" which was prepared 3 months later in a similar manner but using 50 per cent ammonium sulphate. These two healthy-tobacco concentrates were nonstimulatory to normal guinea-pig uteri, as is shown by the records G and H in figure 6. Three such control tests were performed for each extract. The nonerystalline tobacco-mosaic sample used in the experiment was prepared similarly to the healthy-tobacco juice, but by using two successive precipitations with 40 per cent ammonium sulphate. All of the extracts were

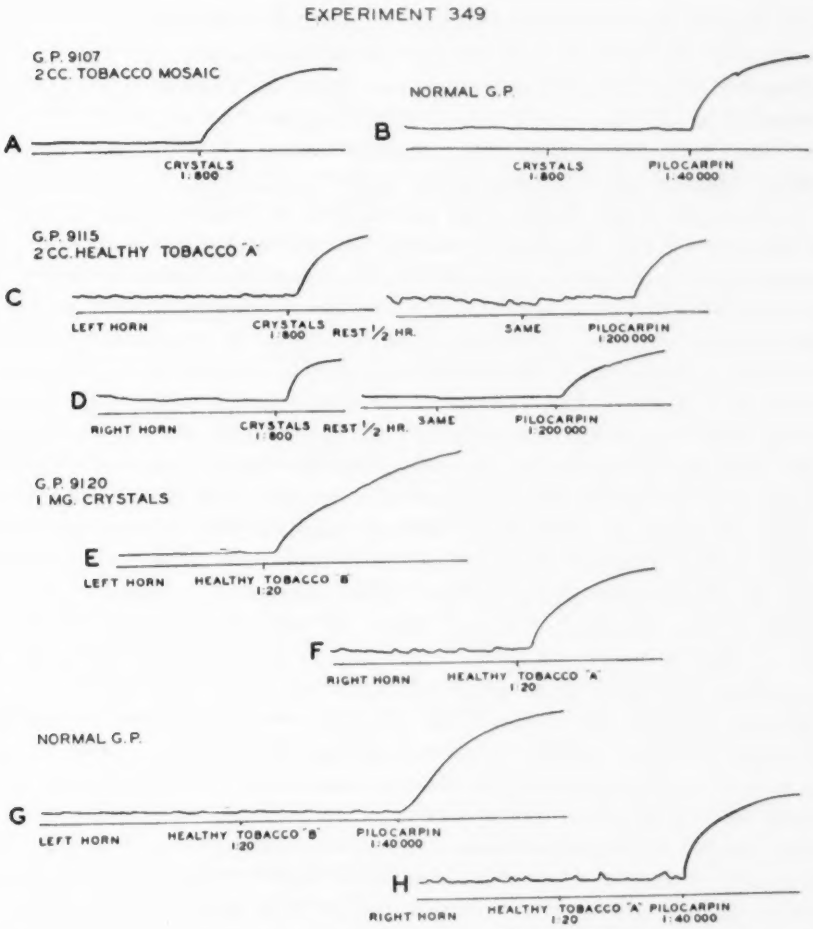

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FIG. 6. Anaphylaxis tests with crystalline tobacco-mosaic protein. Guinea pig 9107, sensitized with whole tobacco-mosaic tobacco extract, reacted with the crystals (Record A) which were shown to be primarily nontoxic (Record B). This reaction must have been due to healthy-tobacco protein, and not to virus, since healthy-tobacco extract sensitized to the crystals (Records C, D), and since 1 mg. of the crystals sensitized to healthy-tobacco protein (Records E, F). Records G and H are toxicity control tests of the tobacco extracts used.

dialyzed against Ringer solution before testing, in order to remove any toxic substances that might have been present.

It was first determined that a guinea pig sensitized with crude juice of a mosaic tobacco plant reacted strongly with a 1:800 solution of the crystalline protein (Fig. 6, record A. The reaction of the right horn is shown; the left horn gave a similar reaction.) Since the virus is not anaphy-

lactic, this reaction is not believed to have resulted from virus in the crystalline protein, but from healthy-tobacco protein associated with the crystals. Subsequent tests showed that the muscles of animals inoculated with mosaic-diseased tobacco juice react with healthy-tobacco proteins, and that after they have been desensitized with healthy-tobacco proteins, they show no residual reaction against the crystals.

Guinea pig 9115 received an inoculation of healthy-tobacco protein, containing no virus. Its muscles reacted with the crystalline material. This is considered to be a true anaphylactic test, since the reaction could not be repeated with the same muscle and, since the crystalline protein was non-stimulatory to normal guinea-pig muscles. Each of two guinea pigs was inoculated with 10 mg. of the crystalline material; a third with 3 mg., and a fourth with 1 mg. Each of the 8 muscles reacted when either of the healthy-tobacco extracts was added to the bath. The reactions of the pig receiving 1 mg. of the crystalline protein are illustrated in figure 6 (Records E and F). Since the reactions were strong, it seemed probable that still smaller amounts of "Crystals I" would sensitize pigs to healthy-tobacco protein. Accordingly, a second experiment of the same type was performed, this time using guinea pigs sensitized with "Crystals I" in dosages of 1 mg. (1 pig), .3 mg. (2 pigs), .1 mg. (2 pigs), .03 mg. (2 pigs), and .01 mg. (2 pigs). The pigs receiving 1 mg., .3 mg., and .1 mg. all reacted strongly with the healthy-tobacco concentrate, even when the latter was diluted 1:1000 (.005 mg. protein per cc.). Some of these tests are shown in figure 7 (Records A and B). Of the two pigs receiving .03 mg. of crystalline protein, one was negative toward healthy-tobacco concentrate, while the other reacted weakly with this juice. Both pigs receiving .01 mg. were negative when tested against the healthy-tobacco concentrate, although highly sensitive to the muscle stimulant, pilocarpin (Records C and D, Fig. 7). The healthy-tobacco concentrate used was not stimulatory to 3 normal guinea pigs. A record from one of these control animals is shown in figure 7 (Record E). Infection tests on *Nicotiana glutinosa* L. showed that the healthy-tobacco concentrate contained no detectable virus. The minimal sensitizing dose of this sample of crystalline protein for reaction with healthy-tobacco protein thus appeared to be in the vicinity of .03 mg. for 200 gm. guinea pigs.

Further anaphylactic tests were performed with the samples of "Crystals II, III, IV, and V." These samples seemed to show a higher state of purity than "Crystals I," as the minimal sensitizing and desensitizing doses required were somewhat greater. The results of all of the tests are grouped in table 1.

Certain of the data given in table 1 may be summarized as follows. From a total of 97 tests, it was evident that there was regular interreaction between crystals from tobacco or tomato and healthy tobacco or tomato. The

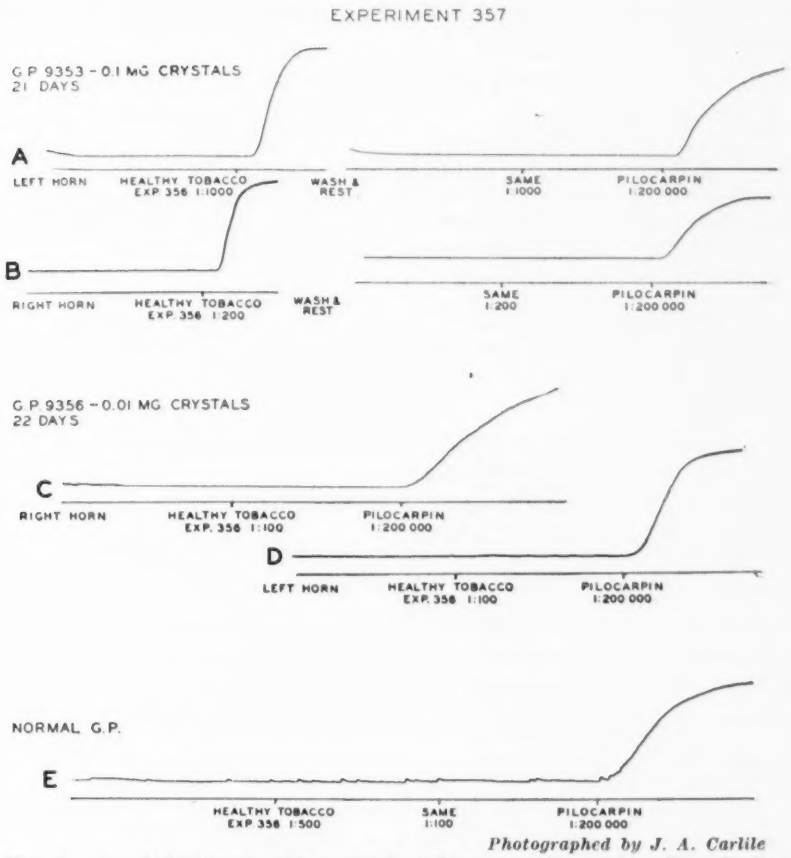

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FIG. 7. Anaphylaxis tests with crystalline tobacco-mosaic protein. Records A and B show that a guinea pig, sensitized with .1 mg. of crystalline protein, reacted strongly with healthy-tobacco concentrate at 1: 1000 (.005 mg. protein per cc.). The reaction was a true anaphylactic test, since the tobacco concentrate at 1: 500 or 1: 100 was nontoxic to a normal guinea pig (Record E), and since a second administration of the tobacco concentrate to a sensitized pig failed to elicit a reacton. A second guinea pig (Records C, D) was not sensitized by .01 mg. of the crystalline protein. The minimum sensitizing dose for this sample of crystals was apparently in the vicinity of .03 mg.

evidence from 43 tests showed a consistent cross reaction between juices of tobacco and juices of tomato. When tobacco-mosaic virus in one host was used for sensitization and the same virus in a second host for desensitization, there was no reaction attributable to the virus, according to 11 tests, exclusive of the tobacco-tomato reactions. Thirty-five negative tests in which animals were sensitized with virus in a given host, desensitized with healthy juice of the same host, and then tested against virus-containing juice, gave further evidence that the virus is not anaphylactic when tested by the Schultz-Dale technique.

TABLE 1.—*Summary of anaphylaxis tests of tobacco-mosaic crystals, including control tests of healthy host juices*

Sensitization	Results of tests	No. of tests
Crystalline tobacco-mosaic protein from tobacco. ("Crystals I, II, V")	With crystalline tobacco-mosaic protein from tobacco, positive at high concentrations, .1 mg. or more usually required to sensitize. Regularly positive with healthy tobacco and healthy tomato at high concentrations.	96
Crystalline tobacco-mosaic protein from tomato. ("Crystals IV")	Positive at high concentrations with healthy tobacco, healthy tomato, crystals from tobacco, and crystals from tomato.	12
Healthy tobacco	Strongly positive even at high dilutions with healthy tobacco and healthy tomato. Positive at high concentrations with crystals from tobacco.	68
Healthy tomato	Strongly positive with healthy tobacco and healthy tomato, weakly positive with crystals from tobacco.	12
Crude juice of tobacco mosaic in tobacco	Strongly positive with tobacco mosaic in tobacco, healthy tobacco, healthy tomato, crystals from tobacco, and latent potato mosaic in tobacco. Negative with virus-containing tobacco, tomato, or crystals from tobacco after desensitization with healthy tobacco. Negative with tobacco mosaic in phlox.	92
1 masked and 4 yellow strains of tobacco mosaic in tobacco	Positive with healthy tobacco and with crystals from tobacco. Negative with virus-containing juices after desensitization with healthy tobacco.	19
Crude juice of tobacco mosaic in tomato	Strongly positive with healthy tobacco and virus-containing tobacco. Negative with healthy and virus-containing phlox. Negative with virus-containing tobacco or tomato after desensitization with healthy tomato.	32
Crude juice of tobacco mosaic in phlox	Negative with healthy and virus-containing tobacco, strongly positive with healthy phlox.	13
Crude juice of tobacco mosaic in pepper	Negative with virus-containing tobacco and phlox.	4

From the experiments reported in table 1, the following conclusions are drawn. Crystals of tobacco-mosaic protein from tobacco and healthy-tobacco juices contain common antigenic material. This applies to materials recently crystallized, as well as to earlier preparations of crystals. The reaction between crystals and healthy-tobacco protein is not regarded as due to a cross reaction between virus and some protein of the healthy host plant that may serve as a precursor of the virus, because the experiments indicate that virus is not anaphylactic when tested by the Schultz-Dale technique. It is, therefore, concluded that there are present in the solutions of crystals proteins that are found in the healthy tobacco plant, and, accordingly, are looked upon as contaminations of the virus in the crystals. The anaphylaxis results thus far available do not warrant conclusions as to the amount of this contamination.

The positive cross reactions between crystals from tobacco and crystals from tomato are not considered to indicate that the virus in these two preparations is anaphylactic and identical, because the experiments show that healthy tobacco and tomato contain common anaphylactogenic material, which has thus far not been completely removed in the process of crystallization. Absorption tests were used to differentiate healthy tobacco and healthy tomato, but as a rule the tests indicate that a large proportion of the protein of these two species is anaphylactically alike.

A comparison of the precipitin and anaphylactic tests with tobacco-mosaic virus leads to the question of why the virus, which is strongly reactive by the precipitin test, fails to show anaphylactic activity even when at a concentration 1000 times as great as the least concentration giving a precipitin test. Although this can not be answered at the present time, it is possible that the large molecular weight of the virus (estimated by Stanley (6) to be of the order of "a few millions") prevents the free diffusion of the antigen into the isolated guinea pig muscle. Protected as the muscle is by several relatively impervious coatings, it is to be expected that the larger the molecule of antigen the greater will be the difficulty of penetration to the probable locale of the antibodies, in the reticulo-endothelial system of the muscle tissue. Hence, the larger the molecule the less activity would be shown anaphylactically. On the other hand, a large molecule would probably be more easily precipitated than a small molecule of protein, other factors remaining constant. The relatively poor solubility of the crystalline protein might likewise be expected to favor precipitation and to interfere with anaphylactic action.

It is evident from the anaphylactic tests that there are present in the mosaic-diseased plant no anaphylactically active antigens other than those allied or identical to the healthy-host proteins, because an animal sensitized with virus-containing plant juice can be completely desensitized with the

juice of the healthy suscept. The possibility often has been considered in the literature that the plant-virus precipitin reaction is not due to the virus but to some serologically active specific by-product of virus activity. The anaphylaxis tests fail to give evidence of the presence of any such specific virus by-product. The experiments do not exclude the possibility that there may be specific, non-anaphylactic products of virus metabolism.

3. *Complement-fixation Tests.* Complement-fixation tests were performed for two purposes, to determine the presence and amount of virus in the crystalline material, and to ascertain whether or not the crystals contained antigenic material present in virus-free tobacco plants that could be demonstrated by this technique. Table 2 gives in condensed form the results of 3 experiments of this type.

TABLE 2.—*Complement-fixation tests*[a] *with crystalline tobacco-mosaic protein*

Antigen	Immune serum	Reaction
1. Crystals	Anti-crystals	Complete fixation to 1: 10,000,000[b]
2. Crude tobacco-mosaic juice	Anti-crystals	Complete fixation to 1: 2,500,000[b]
3. Crystals	Anti-healthy-tobacco	Complete fixation to 1: 660, not above[b]
4. Crude healthy-tobacco juice	Anti-healthy-tobacco	Complete fixation at 1:100, nearly so at 1: 300[c]

[a] *System:* .3 cc. immune serum at 1: 10 or 1: 15 + .2 cc. fresh guinea pig complement at 1: 10 or 1: 15 + 1.0 cc. antigen dilution + 1 hr. at 37° C. + .5 cc. sensitized sheep cells (= .25 cc. 1: 500 or 1: 750 inactive anti sheep amboceptor + .25 cc. 4 per cent washed sheep cells + 1 hr. in cold) + 1 hr. at 37° C.

[b] Dilution of antigen in terms of total protein.

[c] Dilution of antigen solution with original concentration of juice taken as 1: 1.

The writer has previously shown that tobacco-mosaic juices interact by the complement-fixation method at high dilutions, and it is believed that this reaction is due to the virus itself (1). In table 2, reactions 1 and 2 are considered to be due in greatest part to the interreaction of the virus element of the test materials. Reaction 3, however, shows that the crystals also react with healthy-tobacco antiserum, although not at high dilutions. When healthy-tobacco juices are used as antigens with healthy-tobacco antiserum (reaction 4), reaction occurs but only at relatively high concentrations. It is not felt that one should draw conclusions from these data as to the absolute concentration of healthy-tobacco antigen in the crystals, since the quantitative features of the tests are relatively poorly known. The complement-fixation tests are in agreement with the anaphylactic tests in show-

ing the presence in the crystalline protein solutions of non-virus proteins of the healthy tobacco plant. They show in addition that the crystals contain considerable amounts of virus antigen.

4. *Precipitin Tests.* As has been indicated in the preceding pages, the expressed juice of a mosaic-diseased tobacco plant contains two groups of antigens, (a) a complex of proteins such as are present in healthy tobacco plants, and (b) the virus antigen. Precipitin tests have given no indication as yet of a third group, namely, proteins peculiar to the disease but distinct from the virus. It is known from the work of several investigators that the virus antigen of tobacco mosaic is highly precipitinogenic. On the other hand, the healthy-tobacco protein complex has a low order of antigenicity as shown by the precipitin tests. The precipitin test is entirely suitable for detecting virus in tobacco juices, but not well-adapted for detecting healthy-tobacco proteins. Crude virus juice may be diluted 1:250 before the virus precipitin reaction disappears, while the healthy-tobacco protein reaction disappears at 1:10 dilution of the same juice. However, precipitin tests were conducted in order to obtain evidence on the concentration of virus in the crystals, and if possible to shed some further light on the problem of the interreaction of crystals and healthy-tobacco protein. Table 3 gives a summary of the results of one of 10 experiments on this subject.

The data indicate that the crystals contained large amounts of virus antigen. The solutions of crystalline protein above 1:660 dilution failed to react with healthy-tobacco protein immune sera. Below this dilution the solutions of crystalline protein were so opalescent that an end-point reaction could not have been detected had there been one. Crude healthy-tobacco juice, however, reacted with its specific immune serum at no higher dilution than 1:10. It is believed that the failure of the crystals to react with anti-healthy-tobacco sera is due to the low sensitivity of the precipitin test for healthy-tobacco protein and to the opalescence of the more concentrated crystalline-protein solutions that makes it difficult or impossible to detect weak precipitin reactions. Experiments have been conducted that were designed to bring out the presence of a precipitin-inhibiting substance in the crystal solutions, but no evidence of such a substance was obtained. In addition to the tests summarized in table 3, precipitin tests for the purpose of detecting virus also were performed with "Crystals III, IV, and V." Solutions of each of these crystalline preparations reacted strongly with absorbed serum prepared against tobacco-mosaic-virus-containing tobacco juice.

During the course of the anaphylactic investigations, blood was drawn from each of the anaphylactic guinea pigs used, and the sera of these bloods were stored. At the conclusion of this work it was felt desirable to test

TABLE 3.—*Precipitin tests of crystalline tobacco-mosaic protein*

Antigen	Serum		Serum contained antibodies for	Reaction, definitely positive to antigen dilution[b]
	Type	Dilution		
Crystals I	Anti-tobacco-mosaic, absorbed[a]	1:3	Virus only	1:66,000
"	"	1:1	"	1:200,000
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	None above 1:660; see text
"	"	1:1	"	"
"	Normal, absorbed	1:3	.....	None
"	"	1:1	.....	"
"	Normal, not absorbed	1:3	.....	"
"	"	1:1	.....	"
Crystals II	(All tests as with Crystals I, all giving results identical to those with Crystals I, except virus reactions, which were somewhat weaker.)			
Crude tobacco-mosaic juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	1:25,000
"	"	1:1	"	1:75,000
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:10
"	"	1:1	"	1:10
(Normal serum tests as with Crystals I, all negative.)				
Crude healthy tobacco juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	None
"	"	1:1	"	"
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:3
"	"	1:1	"	1:10
(Normal serum tests as with Crystals I, all negative.)				
30 × concentrated healthy tobacco juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	None
"	"	1:1	"	"
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:100
"	"	1:1	"	1:100
(Normal serum tests as with Crystals I, all negative.)				

[a] *Absorption:* 1 part serum added to 2 parts crude healthy-tobacco juice, incubated 2 hrs. at 37° C. + 16 hrs. at 5° C., then centrifuged and precipitate rejected.

[b] Dilution of crystals and crude virus juice expressed in terms of total protein, dilutions of healthy-tobacco juices with original concentration of juice taken as 1:1.

these sera for precipitins, since preliminary experiments had shown that the serum of an anaphylactic guinea pig frequently contains precipitins for the antigen used in sensitization. The sera from 105 of the anaphylactic and normal guinea pigs used in the preceding experiments were tested against the juices of healthy tobacco, mosaic-diseased tobacco, and tobacco diseased with latent mosaic of potato. These tests brought out the following points. Guinea pigs that were sensitized with healthy tobacco and showed strong anaphylactic reactions with healthy tobacco, yielded sera that failed to give evidence of precipitins for healthy-tobacco proteins. As has been pointed out, healthy tobacco proteins are also poor precipitinogens in the rabbit. The sera of animals sensitized with tobacco-mosaic crystals or with the juices of mosaic-diseased tobacco also failed to react with healthy-tobacco juice. The sera of guinea pigs sensitized to either crude mosaic-virus-containing tobacco juice or tobacco-mosaic crystals reacted with virus-containing tobacco juice, provided the sensitizing dose was .3 mg. of protein or more. It frequently happens that individual animals fail to become anaphylactically sensitized when inoculated with doses which sensitize other pigs. In a number of cases guinea pigs were sensitized with 1.0 mg. of crystals and failed in the anaphylactic tests to show sensitivity toward crystals. When sera from such anaphylactically inactive pigs were tested for virus precipitins, they were found to give precipitin reactions as strong as those given by the sera of sensitized pigs. These facts indicate that the anaphylactic test is a much more sensitive test for healthy-tobacco proteins than the precipitin test, and that production of precipitins in sensitized guinea pigs develops independently of the production of an anaphylactic state. In the same animal, tobacco-mosaic virus is a highly active precipitinogen and inactive anaphylactically, while healthy-tobacco proteins are inactive by the precipitin method but highly active anaphylactically. This implies that the mechanisms for the production of the two reactions in the same animal are quite different, even though the same antibodies may be concerned.

SUMMARY

1. The Schultz-Dale method was applied to materials containing several plant viruses, including tobacco-mosaic virus. It was found that the viruses tested gave no anaphylactic reactions. This was shown both by absorbing the muscles with healthy-plant juices prior to testing for virus reaction, and by using as virus hosts for sensitization and testing, respectively, two species of plants so remotely related that the virus was the only common serological element. Healthy-plant proteins, on the contrary, were highly anaphylactogenic. The proteins of healthy tobacco and healthy tomato were very similar serologically.

2. The uteri of animals sensitized with healthy-plant proteins reacted to solutions of crystalline tobacco-mosaic virus protein, and the uteri of animals sensitized with the crystalline protein reacted to dilute extracts of healthy-tobacco proteins. Complement-fixaton tests confirmed the anaphylactic tests in showing cross reactivity between the crystalline protein and healthy-plant protein. The evidence indicates that this cross reactivity is due not to a serological affinity between virus and healthy-tobacco protein, but to the presence in the crystalline material of a contaminating protein serologically allied or identical to protein of the healthy tobacco plant.

3. Precipitin and complement-fixation experiments revealed the presence in the crystalline materials of considerable quantities of virus.

4. Precipitin tests of the sera from sensitized guinea pigs showed that in a given animal tobacco-mosaic virus may be a highly active precipitinogen but inactive anaphylactically, while healthy-tobacco proteins in the same animal may be comparatively inert in producing precipitins but highly active in stimulating anaphylaxis. This implies that the mechanisms of the two reactions are different, although the same antibodies may be concerned in both. It is possible that the molecular size or solubility of the respective antigens underlies this difference in antigenic manifestation.

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RELATIONSHIP OF CLIMATOLOGICAL CONDITIONS TO THE TOBACCO DOWNY MILDEW

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Weather conditions have long been known to influence the prevalence and destructiveness of certain plant diseases, notably those caused by the rusts or by the downy mildews. Our previously recorded conclusions,[2] as to the effect of weather conditions upon the course of the downy mildew disease of tobacco, were based upon observations made during the season of 1933. Subsequent observations, made during the two following years, support the general conclusion, previously drawn, that the progress of this disease is closely circumscribed by meteorological conditions. The correctness of certain other conclusions, especially those dealing with the influence of cloudy, rainy weather in promoting sporulation, also are substantiated by our further experimentation. It appears, however, that such weather may not be favorable, as was once believed, for the wide dissemination of sporangia. Certain other conclusions also must be modified in the light of the additional observations. The results of 3 years' experience have shown that, even though the type of weather may differ each spring, the course of the downy-mildew disease is similar in certain features each year and appears to be predictable. It is the present purpose, therefore, to compare and contrast the pertinent observations and data and to correlate the course of the disease with climatological conditions. Such a treatment should contribute to a better understanding not only of this disease but of other diseases whose progress is conditioned by meteorological factors.

The sources of information from which these correlations have been made are (1) records obtained at field stations, (2) surveys made in representative areas of the State, and (3) climatological data compiled by the Weather Bureau of the United States Department of Agriculture.

Two field stations were established each season at which to make daily observations and to record the progress of the downy-mildew disease. The one, located in eastern North Carolina, was abandoned when the disease became generally prevalent in that locality, and the other was then established in the Piedmont section. A continuous record of temperature and humidity was secured at each station by means of a Friez hygrothermograph placed in a seed bed. In addition, a continuous record was made, in 1935,

[1] Thanks are extended to Dr. P. M. Gross for his criticisms in preparation of this paper.

[2] Wolf, F. A., L. F. Dixon, R. McLean, and F. R. Darkis. Downy Mildew of Tobacco. Phytopath. **24**: 337–363. 1934.

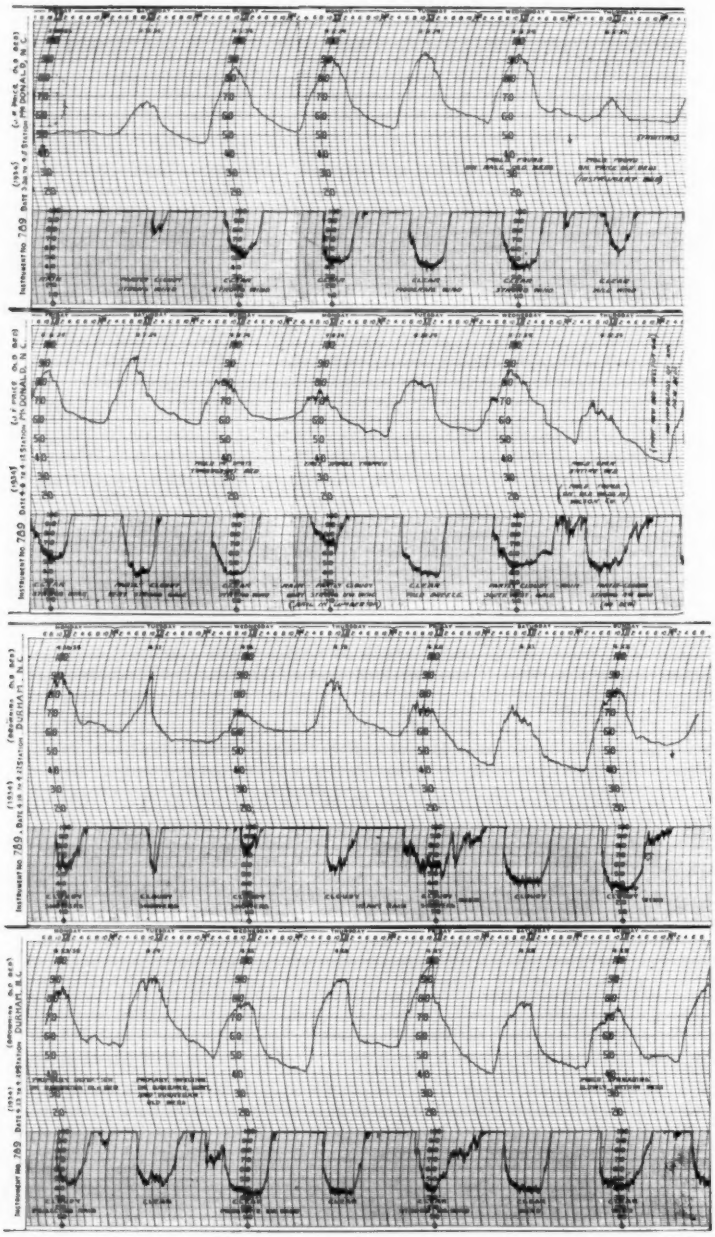

FIG. 1. Upper half, continuous record of the temperature of the air and of the humidity within a seed bed at McDonald, N. C. for the period Mar. 30–Apr. 12, 1934. Lower half, similar record, at Durham, N. C. for the period Apr. 16–Apr. 29, 1934.

of the air temperature just outside the seed bed and of the soil temperature within the seed bed at a depth of approximately ½ in. Records of the air temperatures outside the selected seed beds are not presented. The records, shown in figures 1–5, are, therefore, those of conditions in specific seed beds, but the daily observations took into account the course of the disease in a considerable number of seed beds within the radius of a mile or more of the one containing the self-recording instruments. Our exact knowledge of climatological phenomena and the progress of the disease are, therefore, based upon conditions obtaining in small areas. The data are so interpreted, however, as to make the results widely applicable by supplementing these data with information gained both from surveys of conditions in widely separated areas and from data taken from the reports of the Weather Bureau.

GENERAL STATEMENT OF WEATHER CONDITIONS AND OF THE COURSE OF THE DOWNY MILDEW DISEASE FOR THE YEARS 1931–1935

Weather conditions existing prior to and during the outbreak of downy mildew of tobacco influence the time when primary outbreaks occur, as well as the rate of the subsequent spread and destructiveness of the disease. In order to provide a background for the better understanding of the meteorological data presented in figures 1–5, consideration will be given to the general weather conditions, the progress of vegetation, and the behavior of the disease for the period 1931 to 1935, inclusive.

In 1931. Normal temperatures prevailed throughout January. Since the rainfall was 1.7 inches below normal, and the precipitation previously had been deficient, conditions were favorable for plowing in preparation for spring.

February was generally mild, with temperatures that averaged 1.5° F. above normal. The rainfall was 2.3 inches below normal, as a result of which the stream flow continued low. Small grain and truck crops were adversely affected by lack of moisture.

March was uniformly cold, with a mean temperature 5.5° F. below normal; the coldest March weather within 15 years. Rainfall was well distributed but only about 50 per cent of normal. Much field work was done during the month. Low temperatures retarded plant growth. During the last week of the month downy mildew of tobacco appeared for the first time in seed beds in Louisiana.

The mean temperature in April averaged 1.7° F. below normal. There were several mild periods but no markedly warm spells. Rainfall was 0.8 inch above normal, and was well-distributed, causing overflow of the lower levels of rivers. Crops made good to excellent growth. Downy mildew of tobacco appeared in Georgia during the first week of April, and the first occurrence in North Carolina was reported on April 27. It was then esti-

mated that the disease had been present since the week of April 19–25. Attention also may be directed to its occurrence on large plants in many fields in 11 counties in Georgia, during the last week of April and in early May.

Weather during May generally cold, with average temperature 1.9° F. below normal. Rainfall well-distributed throughout the State and 0.7 inch above normal, resulting in flood stages in many streams. Cold weather retarded growth of all crops. By May 8, downy mildew present in a few seed beds in nearly all counties in southeastern North Carolina. A week later it was reported present in fully half of the beds in this area.

Because of lateness of the outbreak, losses from downy mildew in North Carolina were inconsequential in 1931, the first year of its recurrence in the southeastern United States.

In 1932. January, similar to December, unusually warm. Monthly mean temperature 9.1° F. above normal and almost as high as highest recorded January (1890) temperature. No cold periods occurred during entire month. For 6 days (Jan. 13 to 18) maximum temperature averaged 73° F. and minimum, 54° F., at Lumberton, warm enough to initiate a primary outbreak of downy mildew. Such an assumption is warranted by the observed premature swelling of peach flower buds and the flowering of hardy shrubs and roses. The disease was noted in Georgia on Jan. 21. Rainfall 1 inch above normal; soil too wet for plowing.

February continued exceptionally mild. Monthly mean temperature 6.9° F. above normal, almost the highest mean for that month since 1890. Weather favorable for primary infections occurred at Lumberton from Feb. 10 to 14, when maximum temperature averaged 70° F. and minimum 50° F. The disease was actually reported in this area Feb. 23. It is quite certain that it had progressed to secondary stages before being discovered. An outbreak of downy mildew was reported in Georgia on Feb. 8. Rainfall averaged 1 inch below normal. Eastern counties received 50 per cent of normal amount. Small grain and truck made favorable growth and many shrubs, bulbs, fruit trees, and roses bloomed prematurely.

The mild weather of the 3 preceding months continued until March 6, when a severe storm occurred, followed by a severe freeze that extended to the coast during the following 10 days. Average precipitation 0.4 inch above normal. Strong winds general on March 22, 27, and 28, which were conducive to dissemination of downy-mildew sporangia. The cold wave destroyed the early strawberry crop and damaged truck, grain, and fruits. Numbers of tobacco seed beds were destroyed or severely damaged. The widespread use of sprays for downy-mildew control also adversely affected tobacco seedlings. Monthly mean temperature 3.4° F. below normal.

April temperatures about normal and average rainfall 1.2 inches below

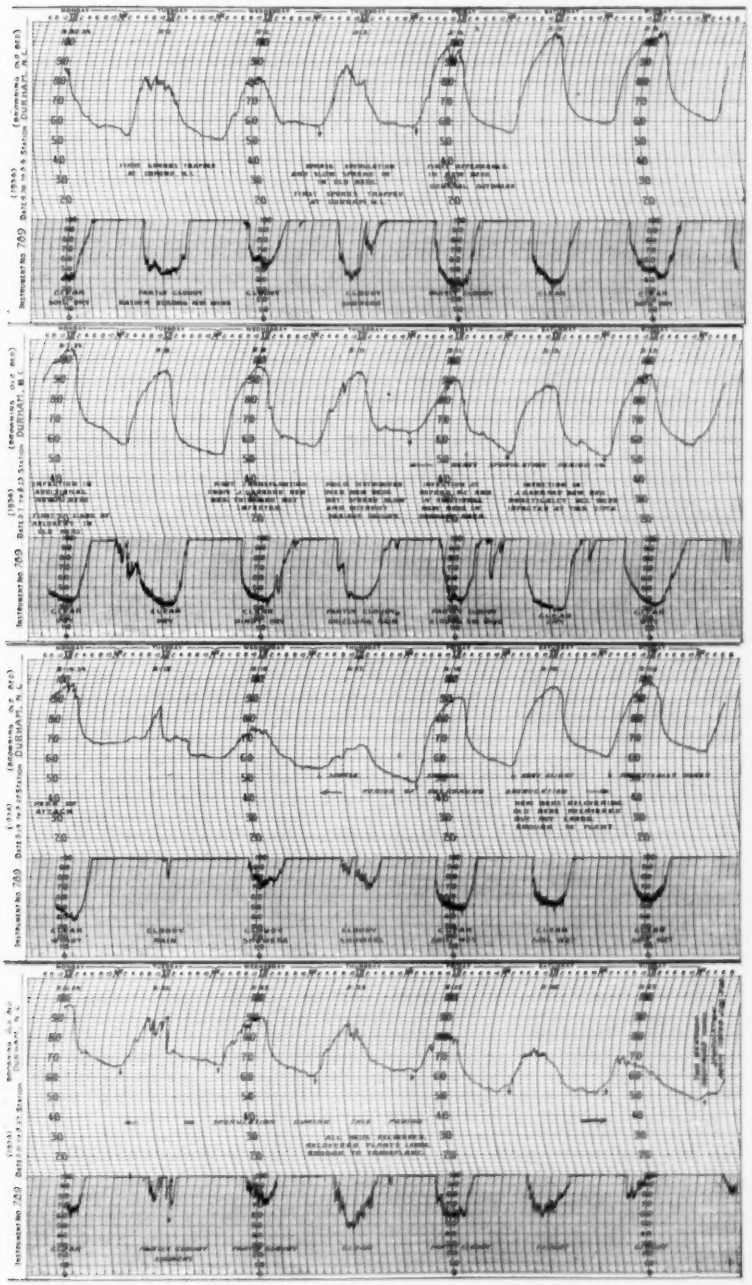

FIG. 2. Continuous record of temperature, and humidity conditions within a seed bed at Durham, N. C. from Apr. 30–May 27, 1934.

normal. The State as a whole received only two-thirds normal rainfall. Conditions favored farm work, but cool weather and insufficient moisture delayed planting 7 to 10 days. Tobacco plants were extremely scarce, and many seed beds were in very poor condition as a result of unfavorable weather, damage by downy mildew, and widespread and injudicious spraying. This resulted in approximately 50 per cent reduction of the 1932 tobacco crop.

May rather cold, averaging 1.0° F. below normal; rainfall averaged 0.4 inch below normal. River stages, below normal. Weather conditions poor to fair for farm work and crop growth. Tobacco varied from poor to fair. Many plants lost in beds and fields because of downy mildew and spray injury. Much replanting, crop made a very late start. During the last week of May downy mildew was noted in the field on plants that were about 2 feet high.

The 1932 tobacco crop was the poorest in quality and quantity in many years. This was attributed to adverse weather conditions, damage by downy mildew and by sprays, insufficient seed-bed areas, and lack of knowledge regarding the handling of seed beds and of transplanting when downy mildew was present.

In 1933. January was abnormally warm; temperature, considerably above normal during about one-half of the month, averaged from +4° to +10° F.; caused abnormal swelling of fruit tree buds and blooming of early shrubs. Precipitation was 0.8 inch below normal; soil, in general, too wet for plowing.

February was characterized by much cloudiness, frequent rains, and moderate temperatures. Two cold waves occurred, one of 2 days,' and the other of 4 days' duration. Monthly mean temperature was +1° F. Precipitation frequent and averaged 0.2 in. above normal. Soil remained wet most of month. Downy mildew reported in Georgia on the 20th.

March temperatures about normal, precipitation 1.5 inches below normal, winds more or less prevalent. A warm period of 5 days occurred at Lumberton (17th–21st) when maximum temperature averaged 75° and minimum 54°. The disease appeared in this section on the 21st. Conditions favored crops and farm work. Tobacco beds generally in good condition at end of month with few, widely separated infected beds in southeastern part of State.

April temperatures, although averaging normal, were variable with prolonged cool rainy periods. Rains frequent and well-distributed. Average rainfall 0.5 inch above normal. Conditions favored good to excellent advance of early truck and fruits. Prolonged cool, rainy periods were conducive to much damage by downy mildew. Producers had greatly increased the area and numbers of beds; no spraying attempted; plants, in general,

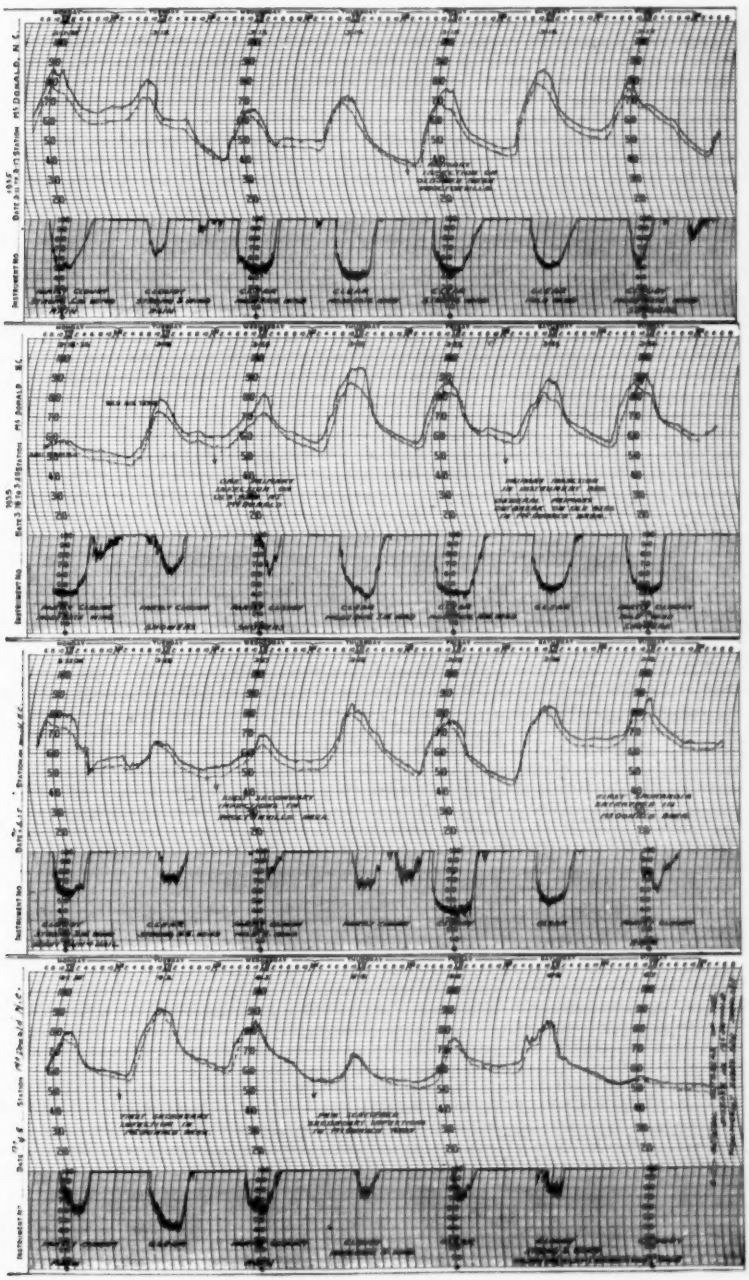

FIG. 3. Record of air and soil temperatures and of humidity conditions at McDonald, N. C. from Mar. 11–Apr. 8, 1935. The upper solid line is record of air temperature, broken line is record of soil temperature.

not transplanted until signs of recovery were seen. These factors finally enabled the contemplated acreage to be transplanted. Considerable tobacco transplanted in the eastern counties during the month. Downy mildew appeared in the Piedmont section on the 7th.

May was the warmest experienced in the State in 37 years. Temperatures, above normal on 25 days, averaged + 3.9° F. for the month. Rainfall above normal (0.4 inch), reasonably well-distributed throughout month. In some parts of the Piedmont, however, rain was needed at the close of the month. Progress of crops, fair to good. Sudden coming of warm weather checked downy mildew, especially in Piedmont section, where it generally appeared May 1. In many localities in this section the disease appeared in nearly all seed beds on virtually the same date. By the 22nd the disease was effectively checked in the more northerly sections, extending to Blackstone, Va.

As mentioned above, downy mildew caused considerable damage to tobacco seed beds during the very prolonged cool, cloudy, rainy periods of April. Producers had benefited by the 1932 experience. They had greatly increased seed bed areas, were beginning to locate the beds in better drained and more exposed sites, had refrained from spraying, and, in general, awaited signs of recovery of the seedlings before transplanting. These improved practices permitted the entire acreage to be planted, and one of the best tobacco crops of recent years was produced. There was no indication that downy mildew was present in the fields nor that the disease caused any bad effects on subsequent growth or quality of the crop.

In 1934. January quite mild until 28th, when most rigorous cold wave in 10 years occurred. Precipitation well-distributed throughout the State but was 1.5 inches below normal. Since the previous months were dry the stream flow was the lowest on record for January.

February was the coldest in the past 29 years, with a mean minimum temperature 6.2° F. below normal. Hard freezes on 8th, 10th, 18th, 27th, and 28th, with sleet and glaze on 25th and 26th, causing severe damage to forests and electric lines. Truck crops, small grain, and tobacco seedlings damaged. Many seed beds reseeded. Precipitation remained below normal; stream flow lowest on record for February.

Weather continued cold throughout March, temperatures averaging 2.0° F. below normal. Hard freeze on 11th and 12th. Precipitation 1.7 inches above normal; only 13 clear days during month. Little opportunity for preparation of soil for spring planting because of cold weather. Growth of tobacco seedlings retarded, by end of month they were 2 to 3 weeks behind normal condition. From March 3–8, at Lumberton, N. C., average maximum temperature 77° F. and the minimum 56° F. No primary infection at this time because previous cold weather had permitted little or no germination

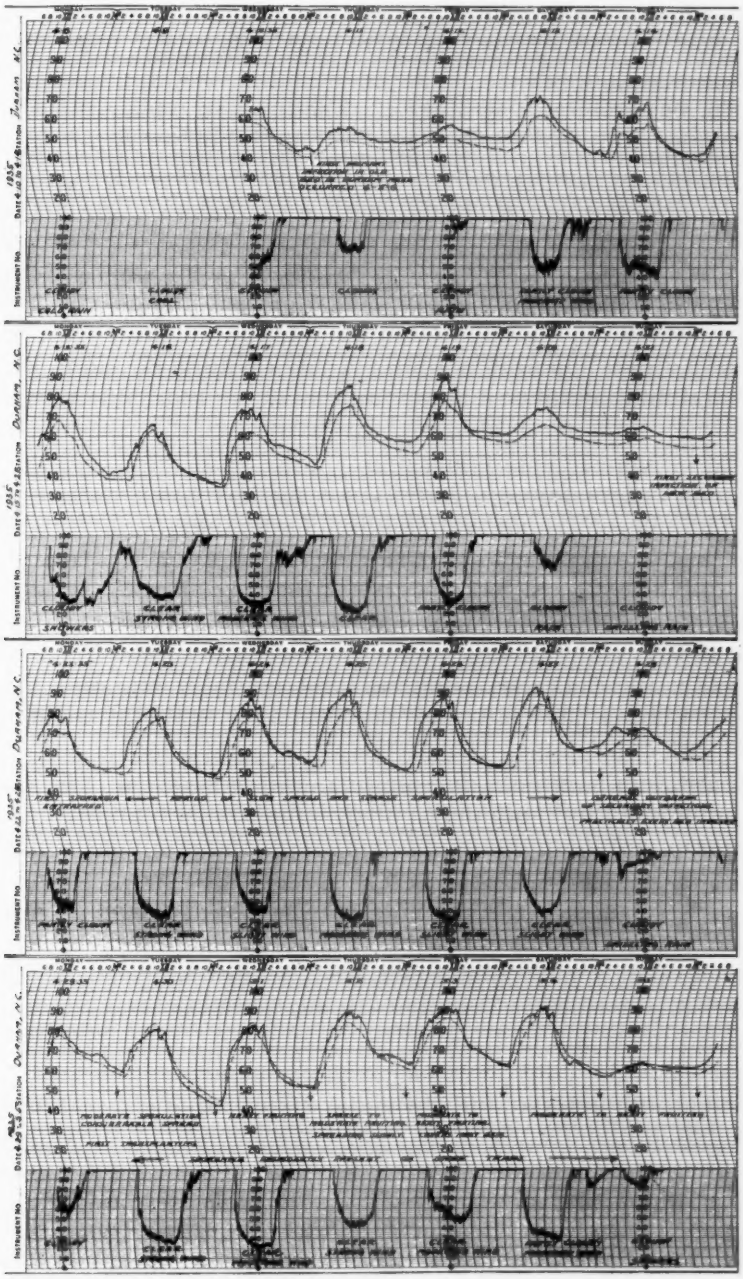

FIG. 4. Continuous record of air temperature, soil temperature, and air humidity within a seed bed at Durham, N. C. for the period Apr. 10–May 5, 1935.

and growth of tobacco seedlings. Minimum temperature for the remainder of the month, except on the 22nd, 27th, and 28th, ranged from 21° F. to 44° F.

April temperature very variable. Month began warm, and soil wet; month closed with cool weather and fields rather dry. Rainfall slightly above normal, poorly distributed. Two rainy periods, April 5–10 and 16–20. Growth of tobacco seedlings approximately 2 weeks behind the normal in all sections. From March 31 to April 8, average minimum temperature, 50° F. at Lumberton, N. C. At McDonald, N. C., self-recording instruments within seed bed showed average maximum of 85° F. and average minimum of 55° F. Primary outbreaks of downy mildew appeared in this area from April 4 to 9. The disease appeared in Wilson county on April 12, and in Durham, Vance, Guilford, Stokes, and Surry Counties on the 23d. Transplanting of tobacco in eastern portions of State about 20 days later than usual on account of downy mildew and unfavorable weather.

Average temperature and precipitation for May was normal except for marked warm, cool, dry, and wet periods. The warmest period came during first week of month and the coolest, during last week. Dry weather of late April extended into first half of May. It was necessary to water tobacco seed beds frequently during this period. Other crops suffered also from lack of water. General heavy rainfall occurred about May 15. Frequent rains and much cloudiness characterized period from 22nd to the end of month. Downy mildew generally prevalent over Piedmont section about May 4; peak of epiphytotic, about May 11. Disease spread slowly, because of dry weather; no serious damage occurred. Sporulation was renewed, however, during cloudy rainy days at end of month on seedlings remaining after transplanting. Less damage occurred in 1934 than in any other year in which the disease has been observed.

In 1935. January generally mild for first 22 days; then cool weather prevailed throughout the remainder of the month. Temperature 10° or more above normal during mild period and as much below normal during cold period. Precipitation averaged 0.5 inch above normal, and was well-distributed. Wet soil and low temperature during latter part of month prevented plowing.

Moderate temperatures during February excepting hard freeze on the last 2 days. Mean temperature was 0.7° F. above normal. Average precipitation 2.8 in. well distributed, was 1.3 in. below the average for a 48-year period. These conditions were very favorable for plowing and for germination of tobacco seed, especially in the southeastern portion of State. The late freeze reduced the stand of tobacco seedlings in a great number of seed beds in the Lumberton area.

March had no freezing temperatures throughout the flue-cured tobacco-

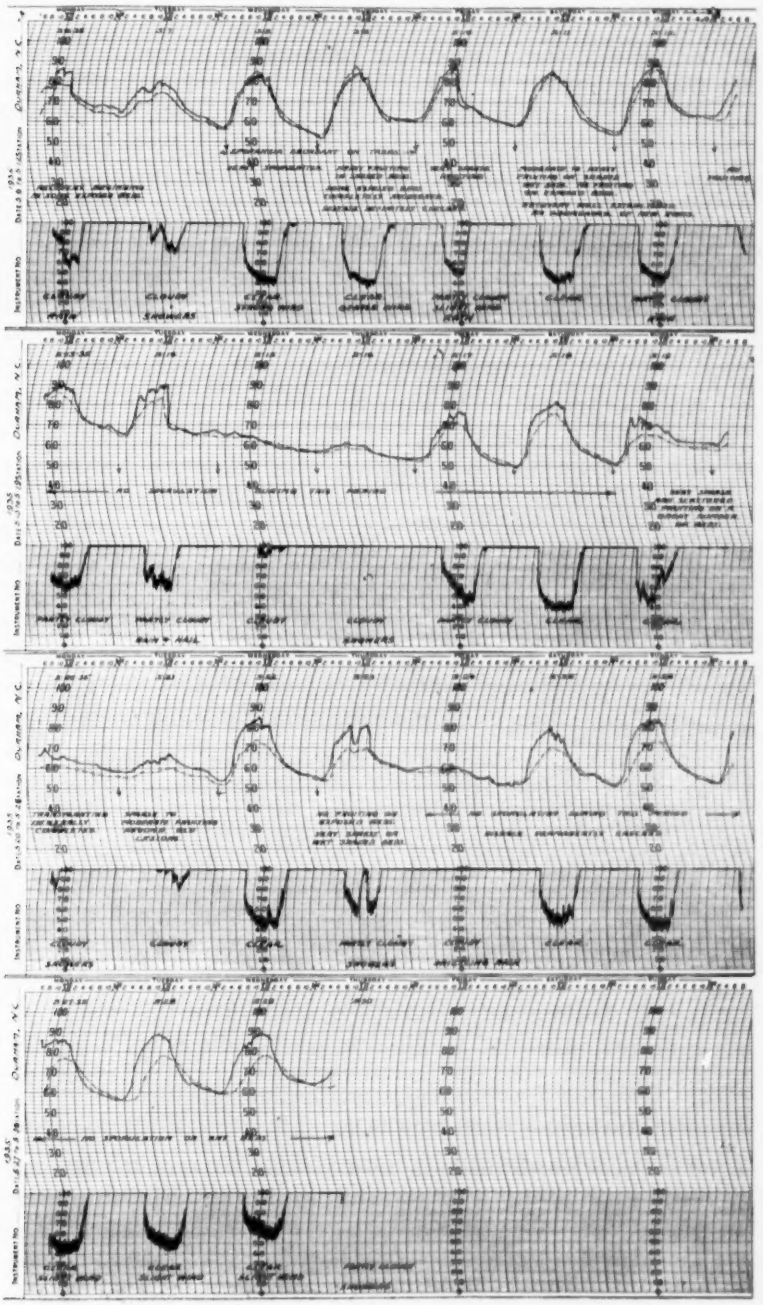

FIG. 5. Continuation of meteorological record at Durham, N. C. from May 6–May 30, 1935.

growing area after the first day. Monthly mean temperature 5.6° F. above normal. March 4–8, warm, maximum temperature averaged 74° F. and minimum temperature 50° F., at Lumberton. This condition is considered favorable for a primary outbreak of downy mildew, but no evidence of disease was found in this section until the 15th. This may be accounted for by the small size of tobacco seedlings at this time, and the thinning caused by the late February freeze. Seeds did not germinate until late February, and the first pair of true leaves was just unfolding. All four leaves were held well off the ground. Another warm period occurred in the Lumberton area March 14–22, when maximum temperature averaged 75° F. and minimum 52° F. Primary outbreaks occurred in this section from March 15 to 23, varying with localized conditions. Average precipitation 0.8 inch above normal. Weather favored rapid advance of vegetation, and season was at least 2 weeks earlier than in 1934.

April, rainy. Cold weather from 4th to 17th. Late April about normal. Average precipitation, 0.6 inch above normal. Low temperatures, frequent rains, and insufficient sunshine retarded growth and interfered with preparation of soil and planting. Some tobacco was transplanted in southeastern part of State before general outbreak on 11th. Some farmers had completed transplanting by 19th; in general, transplanting then only half done. Earliest infected beds then showed signs of recovery. Although nearly every bed in this section was infected, some quite badly, no reduction of acreage was incurred by reason of the disease. Primary outbreaks appeared in Piedmont section on 5th and 6th; general outbreak on 28th. Difficulty in obtaining plants experienced in some parts of the eastern portion of State because of repeated attacks by downy mildew fungus.

May temperatures averaged 1.3° F. below average for past 48 years. May 15th to 26th, cool, with insufficient sunshine for growing crops. Although the number of rainy days during May was above average, total rainfall was below normal in most tobacco-growing areas. Weather in general favorable for transplanting tobacco, and for continued sporulation by downy mildew fungus. Disease only moderately destructive this season in seed beds, but appeared, in several localities in the Carolinas, on large plants in the field.

CLIMATOLOGICAL CONDITIONS IN SEED BEDS AND THEIR INTERPRETATION

The continuous records made in 1934 include those of the air temperature and of the relative humidity inside the seed beds. Those made in 1935 include those of the air temperature outside of the seed bed and of the air temperature, relative humidity, and soil temperature within the seed bed. The air temperatures outside of the seed beds are not shown on the charts, but are essentially the same as those of local Weather Bureau Stations. The

air temperatures within the seed beds usually are slightly in excess of those outside the beds.

The graphs (Figs. 1–5) record the essential features of the course of downy-mildew disease of tobacco as related to climatological factors in 1934 and 1935. Similar records are not available for 1931 and 1932, but a record of a portion of the season of 1933 was obtained, and has been published (*loc. cit.*). Certain features of the disease in 1931, 1932, 1933, mentioned in preceding paragraphs, are known, and will be utilized in substantiation of the conclusions drawn from the data in figures 1–5. It is believed that the interpretation of these data will be simplified if attention be directed separately to such sequential events in the course of the disease and the development of the pathogen as (1) primary infection, (2) secondary infection, (3) sporulation, (4) dissemination of sporangia and the general outbreak of the disease, and (5) the recovery from it. Conditions correlated with each of these events, during each season, will, therefore, be compared and contrasted so far as the available data warrant.

Primary Infection. For the purpose of this report it is arbitrarily agreed that infections are first apparent when sporangia have been produced. Then too, infections are regarded as primary if (1) they occur in seed beds sown the following year on the sites of old diseased seed beds, if (2) sporulation occurs in such beds several (9–17) days prior to sporulation in any new (first-year) seed bed in that locality, if (3) when sporangia are produced, sporulation occurs on the surfaces of the lower leaves of a very few seedlings, and if (4) hibernating oospores constitute the inoculum for these infections. They are regarded as secondary if they occur 9–17 days after sporangia are known to have been present on primarily infected plants in the vicinity. Secondary infections may, therefore, occur (1) in old beds in which sporangia are first produced on primarily infected plants, (2) in new beds near primarily infected old ones, or (3) in old beds in which no primary infections occurred. Secondary infections in primarily infected old beds account for the increase in the number of affected seedlings in those beds.

The incidence of outbreaks, whether primary, secondary, or general, dates from the visible evidence of infection, *i.e.*, the production of sporangia.

The dates of occurrence of primary outbreaks, and also the first recurrence of the disease in 1931, are assembled for convenience in table 1.

The dates on which the disease first appears one year may be very different from those on which it first occurs another year. In explanation it seems reasonable to assume that (1) oospores causing primary infection must occur in the surface layer of soil, and that (2) the most important factor influencing the time of occurrence of primary infection is the maintenance of favorable temperature levels in this surface-soil layer for a sufficient length of time.

TABLE 1.—*Dates of the first occurrence of downy mildew of tobacco in North Carolina, and weather station records of temperatures for warm periods preceding primary outbreaks*

Year	Area	Date of earliest reported downy mildew occurrence	Time of occurrence of warm period	Duration of warm period (days)	Mean max. temp. ° F.	Range of max. temp. ° F.	Mean min. temp. ° F.	Range of min. temp. ° F.
1931	Lumberton	Apr. 27 (probably 19–25)	Apr. 14–22	9	78	70–84	50	43–57
1932	"	Feb. 23 (probably 15)	Feb. 10–14	5	70	62–81	50	43–60
1933	"	Mar. 21	Mar. 17–21	5	75	68–85	54	45–62
1934	"	Apr. 4–9	Mar. 31–Apr. 8	9	82	65–89	50	42–58
1934	Durham	Apr. 23	Apr. 15–21	7	69	56–78	51	43–59
1935	Lumberton	Mar. 20–23	Mar. 14–22	9	75	64–79	52	47–58
1935	Durham	Apr. 4–6	Mar. 28–Apr. 3	7	72	64–84	48	43–52

In 1935, at McDonald, a record of soil temperature of the surface layer was obtained for a 22-day period prior to the primary outbreak in the seed bed containing the recording instruments. Examination of this record of soil temperatures at McDonald reveals the fact that a period of several days, in which the minimum soil temperatures were maintained at or above 50° F., preceded the primary outbreak. It must be borne in mind that this record shows the temperature conditions that obtained in the seed bed in which the instruments were kept. Temperature conditions in other seed beds in the same area most certainly differed, dependent upon such factors as exposure, drainage conditions, type of soil, thickness of seed-bed covers, and protection afforded the beds by surrounding forests. In consequence, not all primary outbreaks may necessarily occur in a given locality on one and the same day.

Unfortunately, primary infection occurred, in 1935, in the selected bed near Durham, prior to the installation of the instruments. The week preceding the outbreak was, however, characteristically mild.

Soil temperatures are not known for 1931–1934. A comparison of the temperatures of the air within the seed beds for March, April, and May, 1935, with the soil temperatures for the corresponding period shows that they rather closely approximate each other. The maximum seed-bed air temperatures average 5° F. higher than the maximum soil temperatures, and the minimum air temperatures average 3° F. higher than the minimum soil temperatures. In 1934, as shown in figures 1 and 2, several days of warm weather preceded primary outbreaks both in the area near McDonald and in that near Durham. The average minimum seed-bed air temperature at McDonald during this critical period was 55° F., and that at Durham was 53° F. The minimum soil temperatures during this period in 1934, as indicated by comparison with temperature conditions within seed beds in 1935, probably were about 50° F.

The records obtained during 1933 do not cover the period preceding the occurrence of primary infection. The temperatures recorded by Weather Bureau Stations for the warm period preceding primary outbreaks in 1933, and 1932, and for the period in which the first outbreak occurred in 1931, show striking similarities. The temperature conditions recorded by the Weather Bureau for these mild periods are compared in table 1, with similar data for 1934 and 1935.

The outstanding phenomenon indicated by these data is that primary outbreaks occur during or following a warm period in which minimum temperatures of approximately 50° F. are maintained for several days. These periods may be characterized as "warm spells." The weather during such spells may be either clear and dry, or cloudy and rainy, without apparent effect on the occurrence of the disease. The primary outbreaks occurring earliest in the season, in the southeastern portions of the State, have taken place during or shortly after clear, bright, warm weather.

Primary infection may not occur during the first warm spell in winter or very early spring. Although seedlings of all ages are known to be subject to attack under controlled environmental conditions, those in seed beds do not become infected until the leaves extend horizontally and the lower ones come in contact with the soil. This may explain why warm periods occurring shortly after the germination of the tobacco seed fail to induce primary infection.

(2) *Secondary Infections.* Examination of figures 1–5 shows that secondary infections were first evident in seed beds near primarily infected ones within approximately two weeks after the first sporulation in primarily infected beds. Our observations show that repeated crops of sporangia are being produced during this period in primarily infected beds, and that a large proportion of the seedlings have become involved.

In 1934, near McDonald, the interval between the occurrence of primary outbreaks and the earliest occurrence of secondary outbreaks in near-by beds was 12 days. The weather for this period was predominantly clear, with moderate to strong winds. The maximum temperature ranged from 63° F. to 94° F. and the minimum from 36° F. to 60° F. The corresponding interval near Durham was 11 days, with the same general type of weather. Maximum day temperature was higher, however, ranging from 75° F. to 100° F. with minimum night temperature from 40° F. to 57° F. The soil generally was rather dry.

In 1935, near McDonald, the interval between the occurrence of primary outbreak and the earliest outbreak in near-by beds was 13 days. The weather was predominantly partly cloudy with some rain, broken by clear days accompanied by mild to strong winds. Maximum temperature ranged from 65° F.–95° F. and minimum from 46°F.–66° F. The corresponding interval, near Durham was 17 days. Weather was extremely variable during this period. Cool rainy periods, cloudy and partly cloudy days, clear days with moderate to strong winds, and periods of drizzling rain were experienced. Maximum temperature ranged from 53° F.–89° F., and minimum temperature from 36° F.–61° F.

The mean maximum temperature for these intervals during the 2 seasons is 78° F., with a range of 53° F.–100° F. The mean minimum temperature is 51° F., and the range 36° F.–66° F. During these periods, the air within the seed beds was saturated for an average of 12 hours per day, and dew remained on the foliage several hours after sunrise nearly every day. Since it is known that infection occurs when viable sporangia are maintained in films of water for several hours, and that saturation conditions prevailed for an average of 12 hours each day, it is concluded that secondary infection may take place during any general type of weather prevalent at this period of year.

TABLE 2.—*Weather conditions during production of sporangia following the general outbreak of the disease*

Year	Period of sporulation following general outbreak (days)	Mean maximum temperature ° F.	Range maximum temperature ° F.	Mean minimum temperature ° F.	Range minimum temperature ° F.	Mean minimum rel. humidity (%)	Range minimum rel. humidity (%)	Av. duration of saturation periods (hrs.)	Range of saturation (hrs.)	General weather conditions
1933	10	81	62– 90	61	52–68	77	54–86	23	12–64	Cloudy, Rainy, Humid and Wet
1934	16	93	67–106	57	47–67	43	28–78	12	6–19	Clear, intense Sunshine, Dry
1935	15	83	64– 92	58	42–64	46	22–80	12	3–17	Consecutive days of clear, windy and cloudy rainy weather

(3) *Sporulation.* In a previous account[2] it was pointed out that the production of sporangia begins in early morning and is usually completed by sunrise, and that it is most profuse during periods of cloudy, rainy weather, when the sky is overcast for long periods. It also is true that sporulation can occur but may be less abundant during other kinds of weather. The period during which sporangia may be produced in any locality may vary in length from year to year, as shown by our later observations. The factors that govern this phenomenon are summarized in table 2.

In explanation of these data it may be recounted that, in 1933, near Oxford, the duration of the period from the general outbreak of downy mildew in new beds until sporangia were no longer produced was 10 days, 7 of which were cloudy and rainy. The average maximum temperature during this period was 81° F., and the minimum ranged between 52° and 68° F. Sporulation was abruptly terminated after a day when the maximum was 90° F. and the minimum was 68° F. In the same year, near Blackstone, Va., almost no sporulation occurred on the morning following a day when the maximum temperature was 97° F. and the minimum night temperature was 66° F. No sporangia were formed subsequently in this area.

In 1934, near Durham, the duration of the corresponding period was 16 days, on 4 of which there was precipitation, and 10 days were clear. Sporulation was sparse on the first 6 days of the period, days that were unusually hot, with maximum temperature of 95° F. or above. This hot spell was followed by 3 partly cloudy days, with maximum temperature near 90° F. and minima of 63°, 53° and 52° F., respectively. Abundant sporulation occurred during these three days. Minimum temperatures ranged from 47° to 67° F. during the remainder of the period and sporulation was sparse.

In 1935, near Durham, the duration of this period of sporulation was 15 days, 7 of which were clear, and on 6 of which it rained. The variability in weather this season was associated with variableness in amount of sporulation. Brief periods of abundant, moderate, and sparse sporulation were intermingled. The maximum temperatures varied from 64° to 92° F. and the minimum from 42° to 64° F.

When consideration is given to temperature conditions prevalent from the advent of sporangia on primarily infected plants to cessation of their development, which includes the sporulation period whose conditions are given in table 2, it is concluded that moderate to abundant sporulation occurs at minimum temperatures ranging from 42° F.–63° F. and sporangia are produced most abundantly at approximately 56° F. If the minimum temperatures are outside this range, either less than 42° F. or greater than 63° F., sporulation is sparse. Little if any production of sporangia occurs above 68° F. or below 36° F.

[2] *Loc. cit.*

As regards the influence of humidity factors on sporulation, attention may again be directed to the data in table 2. During the 10-day interval in 1933, the air within the seed bed remained saturated for periods ranging from 12 to 64 hours in length, or an average of 23 hours. Cloudy, rainy weather prevailed, with a minimum relative humidity of 80 per cent or more for the entire 10 days except for approximately 3 hours. The production of sporangia did not cease at sunrise, but evidently continued throughout certain of these days. As a consequence, the progress of the disease was more rapid than in either of the two following years.

During the 16-day period, in 1934, the periods of saturation of the air within seed beds averaged 12 hours and ranged from 6 to 19 hours. The weather was predominantly clear, with intense sunshine. Minimum relative humidity averaged 43 per cent for the 16 days and varied from 28 to 78 per cent, except for approximately an hour, when it was 80 per cent. Sporangia were sparsely produced, except during 3 partly cloudy days that followed a drizzling rain. The progress of the disease was slower and less damage resulted than in 1933 or in 1935.

In 1935, during the 15-day period, the air within seed beds remained saturated for average periods of 12 hours, the length ranging from 3 to 17 hours. Consecutive days of clear, windy weather and of cloudy, rainy weather occurred. Minimum relative humidity averaged 46 per cent and varied from 22 to 80 per cent. It was 54 per cent or less on 12 of the 15 days. The progress and destructiveness of downy mildew was intermediate between that in 1933 and that in 1934.

From comparison of the data (Table 2) on humidity factors in 1934 and 1935, one might anticipate that the amount of sporulation and the destructiveness of the disease should be quite alike. As a matter of fact, sporulation was more abundant and downy mildew more destructive in 1935 than in 1934. These differences are attributable in part to the large proportion of days with high temperature and intense sunshine in 1934. It may be recalled that a temperature of approximately 85° F. or above, is lethal to sporangia, and that exposure of sporangia to direct sunlight for short periods also is lethal (*loc. cit.*). If comparison be made of humidity factors in 1933 with those in 1934 and 1935, it will be apparent that long periods of saturation and overcast skies favor sporulation.

(4) *Dissemination of Sporangia and the General Outbreak of the Disease.* Air currents are known to be the most important agent for dissemination of sporangia.

The dissemination periods may, for convenience, be divided into two, primary and secondary. The primary period is that interval between the earliest occurrence of sporangia in primarily infected beds and the earliest sporulation in new beds in the same locality. The secondary period extends

TABLE 3.—*Sequence of outbreaks of downy mildew and intervening intervals*

Year	Locality	Dates of primary outbreaks	Elapsed time (primary dissemination period) (days)	Dates of secondary outbreaks	Elapsed time (secondary dissemination period) (days)	Dates of general outbreak	Total elapsed time from first fruiting in primarily infected bed to fruiting in all beds (days)	Date when sporangia first trapped
1933	Oxford	Apr. 7				Apr. 30–May 2	25	Apr. 25
1934	McDonald	Apr. 4–5	12	Apr. 16	12	Apr. 28	24	Apr. 9
	Durham	Apr. 23	11	May 4	10	May 14	21	May 3
1935	McDonald	Mar. 20–23	13	Apr. 2	9	Apr. 11	22	Mar. 31
	Durham	Apr. 5–6	17	Apr. 22	6	Apr. 28	23	Apr. 22

from the date of the secondary outbreaks to the date of the general outbreak. The inoculum disseminated during this secondary period originates both in primarily and secondarily infected beds, and is, therefore, greater in amount than occurs during the primary period.

These periods of dissemination and other pertinent facts for 1933, 1934 and 1935 are shown in table 3.

In 1935, near McDonald, the primary dissemination period lasted 13 days. Sporangia were first entrapped on March 31. Infections probably arose from inoculum disseminated on March 25–27, when windy weather prevailed. A few seed beds at widely separated points throughout the area evidenced infection on April 2. The secondary period of dissemination lasted 9 days and terminated on April 11. The weather was cool and rainy, interspersed with dry, windy days.

The primary dissemination period near Durham lasted 17 days. A few plants in a small number of beds evidenced infection at the end of this period. The fact that no sporangia were found on the spore traps during this period might be interpreted to indicate that conditions were not favorable for dissemination. There were, in fact, few sporangia to be disseminated because low night temperatures interfered with their production. On April 20, 21, and 22 abundant sporulation took place and the 5 clear, windy days that occurred in succession thereafter were ideal for dissemination. On April 28, the day following these 5 days, the general outbreak of the disease occurred. Several small groups of plants in every bed evidenced infection on this date.

As shown by tests with spore traps, sporangia were being disseminated in abundance near Durham, in 1935, from April 30 to May 6 and May 8 to 9. The weather was clear with gentle winds, and sporangia were being produced in abundance during these two intervals.

In 1934, near McDonald, the primary period of dissemination terminated on April 16, 12 days after the primary outbreak. Sporangia were first entrapped on April 9. During this period (April 4–16), temperature and humidity conditions were conducive to moderate production of sporangia, and protracted winds, varying in intensity from a mild breeze to a gale, facilitated transport of sporangia. Consequently, a large number of beds were infected at the end of the primary period. The weather of secondary period, April 17 to 28, favored dissemination but was unfavorable for sporulation.

In 1934 the primary dissemination period near Durham terminated May 4, and included an interval of 11 days. Sporangia were first entrapped 10 days after the primary outbreak. The maximum temperature of 100° F. on April 27, probably was lethal to sporangia that may have formed early that morning. The 6 days that followed, with an average maximum temperature

of 82° F. and an average minimum of 51° F. favored sporulation. The then prevalent winds favored dissemination. As a result, groups of diseased plants were found present in about 80 per cent of the beds examined on May 4. The area occupied by diseased plants in these beds increased slowly during the next 10 days, and the infections had meanwhile become evident in the remaining seed beds near Durham.

In 1933, near Oxford, the first dissemination period lasted from April 7 to approximately April 30. Sporangia were first entrapped on April 25. The continuously cloudy, rainy weather of the week from April 15 to 21 was excellent for abundant production of sporangia but inhibited their transport. The following week favored both production and dissemination. In consequence, downy mildew appeared in many seed beds on April 30, and the general outbreak occurred two days later. The primary and secondary periods of dissemination were, therefore, fused because of the lack of disseminating conditions during the normal primary dissemination period.

It is, therefore, apparent that in each of these seasons an interval of about 3 weeks elapsed between the occurrence of sporangia on primarily infected plants and the general outbreak of the disease. This apparently is not fortuitous but is presumably related to the fact that a period of about 7 days is required for the completion of the cycle of the sporangial stage. On this basis sporulation on new beds might be expected to occur one week after that on primarily infected beds. It appears, however, that infection from this inoculum is confined to seedlings near the primarily infected seedlings and in the same bed. The inoculum produced on these additional plants is sufficient to initiate the secondary outbreak at the end of another week. This accounts for the occurrence of secondary outbreaks about 2 weeks after primary outbreaks. The general outbreak occurs about a week later, due to the greatly increased quantity of inoculum disseminated from widely distributed sporulating beds. Weather conditions may so affect sporulation and dissemination as to prevent secondary outbreaks from occurring about 2 weeks after primary outbreaks, as was the case in 1933; or, on the other hand, they may be such as to cause the disease to appear in a large number of beds at this time, as was the case in 1934.

It is concluded from these data that sporangia are disseminated during windy weather, which may be either clear or cloudy. Continued rainy weather, combined with long periods of saturation, although conducive to abundant sporulation, inhibits dissemination. The greatest quantity of inoculum is disseminated when abundant sporulation occurs during windy weather, or when windy weather follows immediately after heavy sporulation periods. If prolonged periods of high temperatures, intense sunshine, and drying winds obtain, few sporangia are disseminated, because such weather results in a dearth of inoculum. Only a few sporangia transported under such conditions are capable of producing infections.

(5) *Recovery from Downy Mildew.* The time required for downy mildew of tobacco to run its course varies from year to year, a fact no doubt related to the meteorological factors that modify sporulation. When the weather is unfavorable for production of sporangia, the lesions may appear as yellow flecks. When sporulation is sparse, there may be successive scant crops of sporangia on new tissue or at the margins of old lesions on several successive mornings. When sporulation is abundant, one or two crops of sporangia are produced, after which the invaded tissues collapse rather quickly. Our observations show that plants begin to recover when sporulation ceases. A summary of weather conditions that prevailed during periods in which sporangia were not produced in 1933, 1934, and 1935 is shown in table 4.

It may be noticed that in the areas considered in table 4 the period extending from the general outbreak to the beginning of recovery lasted 10 days in 1933, 16 days in 1934, and 15 days in 1935. In 1933 the average minimum night temperature during the period of recovery was unfavorable for sporulation. In 1934, both the night temperature and relative humidity conditions were favorable during this period. In 1935 a period of sparse sporulation of 5 days duration interrupted the 2 periods in which sporangia were not produced. During both periods the average maximum temperature approximated 80° F., the average minimum temperature approximated 57° F., and the average duration of periods of saturation was 18 hours. On the basis of the conclusions previously drawn regarding the influence of climatic factors on sporulation, one would have expected the weather conditions, in both periods, to favor abundant production of sporangia.

It seems reasonable to conclude from the data shown in table 4 that the initiation of recovery from downy mildew is not primarily conditioned by weather. Further evidence in support of this conclusion is afforded by the fact that seedlings in one end of a bed may be recovering while the disease is not visibly present on those in the other end, and while those intermediately located are suffering intermediate stages of the disease. This condition is most noticeable in primarily infected seed beds. No satisfactory explanation for this behavior can be given at this time. The factors that initiate recovery, however, seem not to be external but internal as regards the seedlings. If immunity is conferred to tobacco seedlings as the result of downy mildew infection, it must be regarded only as temporary. In 1935, for example, sporulation occurred from May 19 to 23, on plants on which the production of sporangia had ceased for a period of a week prior. The reinfection of seedlings after they are well on the way to complete recovery has been noted by various investigators. The fact is equally well-known that plants are subject to attack after having been transplanted sufficiently long to have become well-established in the field.

Although weather conditions are not of primary importance in initiating recovery, they can modify the rate of recovery. Warm, clear days, followed

TABLE 4.—*Weather conditions during recovery from downy mildew*

Location and year	Interval from general outbreak to first cessation of sporulation (days)	Periods of no sporulation	Mean max. temp. (°F)	Range max. temp. (°F)	Mean min. temp. (°F)	Range min. temp. (°F)	Mean min. humidity (%)	Range humidity (%)	Average duration of saturation (hrs.)	Range saturation (hrs.)
Oxford, 1933	10	May 12–16	91	87–94	68	67–69	87	80–92	21	18–44
Durham, 1934	16	May 20–27	88	74–98	60	51–66	57	45–70	13	10–16
Durham, 1935	15	May 12–19	79	61–90	58	50–66	52	35–90	18	13–37
		May 23–30	81	60–89	56	51–64	45	38–52	18	13–38

by warm nights inhibit sporulation, are lethal to sporangia, and promote the growth of the tobacco seedlings. There is thus a basis for the impression commonly held that warm sunny weather causes infected seedlings to recover.

GENERAL SUMMARY

This report deals with climatic conditions that occurred in North Carolina in 1931–1935 as related to the course of the downy-mildew disease of tobacco.

The weather for each year is presented by months, as a background for an understanding of differences in time of occurrence and course of the disease.

Primary outbreaks do not occur on corresponding dates in successive years. They occur during or immediately after periods of warm weather, (1) when the minimum temperature of the surface layer of soil has been maintained at or above 50° F. for several days, and (2) when the seedlings are of sufficient size for the lower leaves to come in contact with the soil. They occur irrespective of the character of the sky or of rainfall.

Secondary infection may occur during any general type of weather prevalent at this season of the year, because dew accumulates on the leaves of the seedlings each night. Secondary outbreaks occur approximately 2 weeks after primary outbreaks, and are conditioned to some extent by the weather as it affects sporulation and dissemination.

Sporulation is favored by long periods of saturation at times when the sky is overcast. Abundant sporulation occurs within a range of temperature from 42° F. to 63° F. and is most abundant at 56° F. If the temperature is outside this range, either less than 42° F. or greater than 63° F., sporulation is sparse. Few if any sporangia are formed above 68° F. or below 36° F. Maximum temperature above 90° F., accompanied by intense sunshine, inhibits abundant sporulation even when night conditions are favorable.

Windy weather, either clear or cloudy, is favorable for the dissemination of sporangia. The greatest amount of inoculum is disseminated when windy weather occurs during, or immediately after, periods of abundant sporulation. The general outbreak of downy mildew in any locality occurs about 3 weeks after the primary outbreak. The primary period of dissemination is approximately 2 weeks long and the secondary period approximately 1 week.

Weather conditions are not of primary importance in initiating recovery. Recovery appears to be initiated by factors within the plant. Tobacco seedlings recover most rapidly, however, during warm clear days, and warm nights.

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FURTHER STUDIES ON DOWNY MILDEW OF TOBACCO

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INTRODUCTION

Downy mildew is a disease of tobacco seedlings of major importance to all growers of flue-cured tobacco within the United States and to others in portions of the areas devoted to the culture of Burley tobacco. The results of our investigations dealing with certain features of this disease and with its causal agency have been stated and interpreted in previous reports (16, 6, 18, 19). Additional experimental data, secured during the past year, and observations made in the field during the past 4 years, are here assembled to supplement the present knowledge of this disease. These data and observations partly confirm our previous findings and are corroborative of conclusions previously drawn.

Evidence in an earlier report (6) led to the conclusion (1) that oospores serve as inoculum for primary infections and (2) that seed beds sown on the sites of beds devoted to tobacco seedlings the preceding year constitute the loci of primary infection for downy mildew. The probable correctness of these conclusions is supported by the fact that, in 1934, the disease was found to develop earlier in seed beds located on sites occupied by beds during the previous year than in those sown on new sites. The earliest development of the disease in every locality occurred, in every instance, in beds sown on the sites of old beds. Furthermore, sporulation in primarily infected beds occurred prior to the time that sporangia could be entrapped from the air in the same locality. Since the probability remained, however, that the findings relating to the initiation of downy mildew in 1934 might not be duplicated in another season, or in other areas, the investigations were continued throughout the season of 1935. The same methods of investigation already described (6) were followed in this portion of the work.

INITIATION OF PRIMARY INFECTIONS

Two field stations were established this season, where daily observations on the initiation of downy mildew and on the progress of the disease were made. One was located in Robeson County, near McDonald, North Carolina, and the other in Durham County, near Durham. These observations,

[1] The writers gratefully acknowledge the sustained interest of Dr. P. M. Gross in the investigations of downy mildew of tobacco, and are appreciative of his criticisms during the preparaton of this report.

begun March 1 and continued till May 31, were supplemented, as much as time permitted, by surveys involving areas in Horry County, South Carolina, and in Wake, Granville, Orange, and Vance Counties, North Carolina. When downy mildew became generally prevalent in the environs of McDonald, observations in that locality were discontinued. Consideration was given not only to the dates of appearance of the disease in beds on old sites in comparison with those on new sites, but also to the prevalence of air-borne sporangia as indicated by spore traps (Fig. 1, A), and by the time of occurrence of secondary infections in the vicinity of primarily infected beds (Table 1).

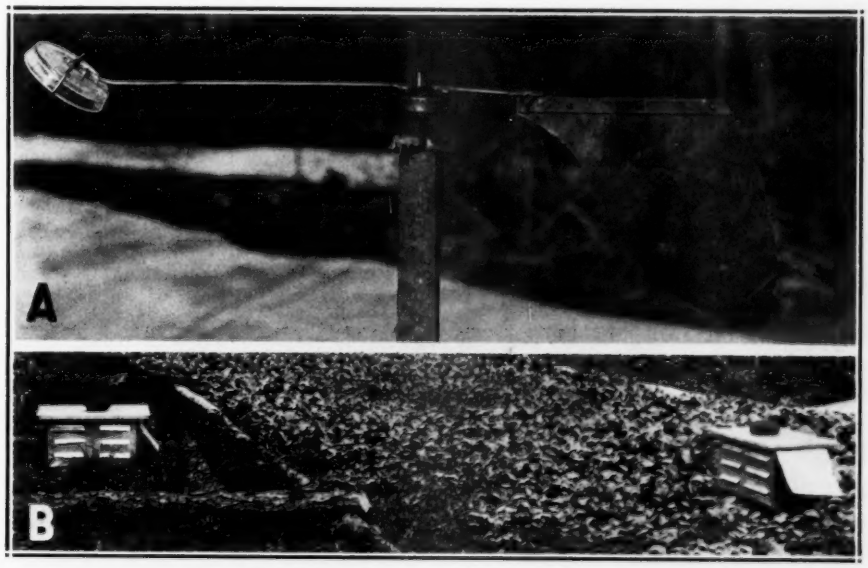

FIG. 1. A. Spore-trap mechanism, consisting of a roller bearing and a shaft at one end of which the dishes were attached and at the other a vane. By this means the adhesive inner surface of the dish was continuously directed toward the wind. B. Ventilated shelters for the protection of the Friez instruments used in making continuous records of soil temperature, air temperature, and air humidity. The shelters were insulated with Cellotex pads.

Attention first should be directed to the fact that, without exception, the earliest infections again occurred in beds located on sites occupied by diseased beds during the preceding year. Among the 114 beds on old sites, under observation in 1935, primary infection occurred in 31. In 1934, primary infection occurred in 37 of the 106 old beds. In explanation of the proportional differences in number of primary infections in these two seasons it may be pointed out that in the Durham area a special effort was made in 1935 to thoroughly sterilize, by burning, all old bed sites that

TABLE 1.—*Summary of data on the effect of site of seed beds upon the incidence of downy mildew, and on the occurrence of air-borne sporangia*

Observations	Areas			
	Robeson Co., N. C.	Horry Co., S. C.	Durham Co., N. C.	Total
Beds on old sites, number examined	61	30	23	114
Beds on new sites, number examined	59	16	49	124
Beds on old sites, area (sq. yds.)	6,480	2,040	4,210	12,730
Beds on new sites, area (sq. yds.)	7,420	4,160	8,130	19,710
Beds on old sites, number of primary infections	14	12	5	31
Beds on new sites, number of primary infections	0	0	0	0
Beds on old sites, dates of primary infection	Mar. 15–23	Mar. 15–20	Apr. 4–6	
Beds on new sites, date of earliest occurrence of downy mildew	Apr. 2	Mar. 28	Apr. 22	
Date when downy mildew was generally prevalent	Apr. 11	Apr. 11	Apr. 28	
Number of spore-traps exposed	288		364	652
Area of surface of spore-traps, sq. cm.	16,416		20,748	37,164
Number of days on which spore traps were exposed	38 (Mar. 1–Apr. 8)		61 (Apr. 1–May 31)	91
Number of days on which sporangia were entrapped	4		40	44
Date on which sporangia were first entrapped		Mar. 31	Apr. 22	

were employed. The percentage of the beds on old sites that evidence primary infection in spring is, however, of less significance than the fact that primary infection occurs in such beds, and that infections in beds on new sites did not occur prior to the outbreak of the disease in old beds in that locality.

The meteorological conditions of 1935 were more favorable than those of 1934, for the earlier development of tobacco seedlings and of other vegetation. Primary infections of downy mildew were evident approximately 2 weeks earlier than in 1934, and could be found in any locality when the flowering dogwood, *Cornus florida*, came into blossom. The dates of primary outbreaks in Robeson County and the contiguous area in Horry County extended over the period March 15–23; and in Durham County, April 4–6; the corresponding dates, in 1934, for these areas, were April 4–9, and April 23–24, respectively. Our observations show that primary infections were first evident, on March 29, in Greene County and in southern Wake County, areas in which the advance of the season is intermediate between that in the vicinity of McDonald and of Durham. This indicates that, concomitant with the advance of the season, there was

again, as in 1934, a northward progression in the occurrence of primary outbreaks.

Downy mildew first occurred, this year, in the Georgia area, on March 7. Since its earliest occurrence in South Carolina and North Carolina was only a week later, there would appear to be little likelihood that inoculum from Georgia initiated the outbreaks in these States.

Emphasis also should be placed upon the highly localized character of primary infections, previously pointed out (6), in differentiating between the appearance of a primary and a secondary infection. In primary infections a crop of sporangia may be found upon the lower surface of a single lower leaf of a lone seedling or upon the lower leaves of a group of 3 or 4 plants. The discovery of primary infection in a seed bed of several hundred square yards can reasonably be said, therefore, to be difficult. When, however, sporangia on primarily infected plants have given rise to secondary infection, and seedlings in an area a foot or more in diameter have become affected, the locus of infection is readily apparent.

In a given seed bed there were usually very few (1 to 5) loci of primary infection. It is not known why primary outbreaks are so highly localized rather than widespread, in view of the probability that myriads of oospores must be formed in every seed bed, and, on decay of the leaves, must be liberated in the soil of that bed. Many of them may be destroyed by biotic agents. Evidence will be presented subsequently to show that only a small proportion of the oospores retain their ability to germinate. It is probably for these reasons that a few, infected seedlings, or but one, may be present in a bed containing thousands of seedlings.

Another important feature of the observations summarized in table 1 is the intervals that elapsed between the time that primary infections are apparent and the earliest outbreak of downy mildew in near-by seed beds. This interval was about 10 to 14 days. It may be pointed out also that an interval of approximately 3 weeks elapsed in both 1934 and 1935 between the date when the first crop of sporangia was produced in primarily infected beds and the date when the disease was beginning to be generally prevalent in that locality. Both in the earliest occurrence of infection in secondarily infected beds and in the general outbreak of downy mildew, the fairly well defined periods found are undoubtedly due to the fact that the development of the sporangial stage (including germination, penetration, incubation, infection, and sporulation) usually requires approximately a week. The number of sporangia borne on primarily infected plants is small. As a result of secondary infection, however, in these primarily infected beds, the inoculum increases so that, within 10 to 14 days, an occasional seed bed near at hand is infected. The amount of

inoculum originating in these scattered beds is sufficient to cause a generalized outbreak after an additional week, and the disease will, soon afterwards, have attained epiphytotic proportions.

The spore traps consisted of shallow dishes (Fig. 1, A) whose inner surfaces were coated with glycerine. Sporangia were entrapped on 44 days of the 91 on which spore traps were exposed. The series of dishes exposed from April 22–May 31 showed that dissemination was occurring throughout the entire period. At 2 periods, namely, April 30–May 5 and May 8–9, sporangia were abundantly present in the air. The production of abundant crops of sporangia on the seedlings occurred during these intervals. Sporangia of other species of downy mildew were present on the plates throughout most of April and May. This was expected to occur, since the following species of downy mildew were collected on weeds growing within the beds or in the area closely surrounding them: *Peronospora alsinearum* Casp. on *Cerastium viscosum* L., *P. halstedii* Farl. on *Ambrosia artemisiifolia* L., *P. geranii* Pk. on *Geranium carolinianum* L., *P. parasitica* (Pers.) Fr. on *Cardamine pennsylvanica* Muhl., *Basidiosphora entospora* Roze et Cornu on *Erigeron canadensis* L., and *Bremia lactucae* Regel on *Lactuca canadensis* L. Cabbage seedlings commonly are grown along the border of tobacco seed beds in North Carolina and generally are parasitized by *Peronospora parasitica*. The sporangia of all of these, except *P. alsinearum*, can fairly certainly be differentiated from those of *P. tabacina* on the basis of size and shape. The significant result of the experiments with spore traps is that sporangia were entrapped, regardless of the particular species; that those of *P. tabacina* were most abundant on the dishes at times when sporangial production was most abundant on the tobacco seedlings, and that sporangia were not present in the air until a considerable period after primary outbreaks of downy mildew had taken place.

GERMINATION OF OOSPORES

Observations on germination of the oospores of the Peronosporaceae are limited. They appear to be confined, in the case of the genus Peronospora, to those of McKay (15) in his studies of the onion mildew, *P. schleideni* Ung. He placed affected leaves containing large numbers of oospores in a box that remained exposed in a garden. This material was tested from time to time by placing a portion of it in water, with the result that after 4 years an occasional oospore germinated. Five months later about 1 per cent of a lot of oospores that had been kept in water for 11 days were noted to have formed a stout tube that penetrated the enveloping folds of the persistent oogonial wall.

In testing the germination of oospores of *Peronospora tabacina*, decaying diseased leaves, collected in May, 1933, were placed intermittently in cold storage and in the laboratory. In the following December an occasional oospore produced a germ tube when bits of this material were macerated and kept in drops of water on microscopic slides. Other oospore-bearing material was collected in 1934, a portion was air-dried and kept in the laboratory; another portion was mixed with sand in a porous earthen vessel and then buried in the soil of an old bed. During the winter and spring of 1935, samples from each portion were taken periodically and maintained in drops of water to permit of germination at temperatures ranging from 36° F. to 75° F. Since less than a dozen oospores were observed to germinate as a result of these tests in which approximately several thousand oospores were employed, nothing definite is known about the environmental factors governing germination.

The first evidence of germination is the uniformly granular condition of the entire content of the oospore. The germ tube emerges through a cleft in the brown inner membrane and the clear outer oogonial envelope, or else it perforates this envelope (Fig. 2). The content of the germ tube is at first brown and granular, and of the same appearance as that of the oospore. At the tube continues to elongate, the contents become less

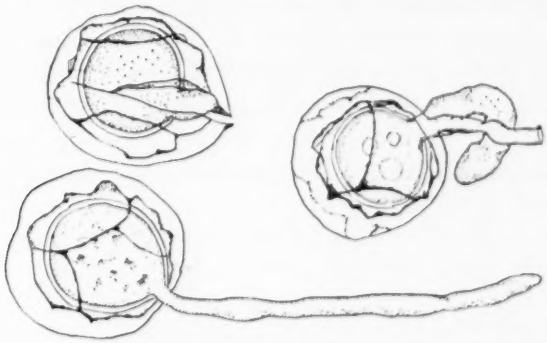

FIG. 2. Stages in germination of the oospores of *Peronospora tabacina*.

densely colored. The maximum length of this tube is about 4 times that of the diameter of the oospore.

The logical assumption, previously made (6), and supported by the current season's experiments, is that the oospores of *Peronospora tabacina* hibernate in the soil and constitute the inocula for the primary infections of tobacco seedlings. This assumption becomes the more cogent in view of the fact that the oospores have been observed to germinate. It is entirely in accord, moreover, with the opinion of mycologists generally as to the rôle of oospores in the life cycle of downy mildews. This is

borne out by the observations on downy mildew of grapes, *Plasmopara viticola* (B. et C.) Berl. et de Toni, (8), the downy mildew of cucurbits, *Peronoplasmopara cubensis* (B. et C.) Clinton (11, 14), the downy mildew of grasses, *Sclerospora graminicola* (Sacc.) Schroet. (9, 10), the downy mildew of onions, *Peronospora schleideni* Ung. (13, 15) and that of spinach, *P. effusa* (Grev.) Ces. (12). Unfortunately, penetration of tobacco seedlings by germ tubes from oospores of *P. tabacina* and the subsequent stages of infection have not been observed. It is, of course, highly desirable that these phenomena be observed and described, but the chances of their accomplishment appear remote when it is remembered that primary infection involves a few seedlings among a population of thousands, and apparently arises from only one or a few germinating oospores in seed beds in whose soil multiplied thousands of oospores are probably present.

SIZE OF THE REPRODUCTIVE STRUCTURES OF PERONOSPORA TABACINA

The measurements of the reproductive structures of *Peronospora tabacina,* as recorded by various investigators, do not agree. In no case have these records been based upon a sufficiently large number of measurements. For this reason a relatively large number of measurements of sporangia, oogonia, and oospores have been made, and the results have been assembled graphically in figures 3 and 4. Each graph shows the range in size and percentage of each size. Using freshly-formed sporangia, approximately 1,100 measurements of each dimension were made. More than 600

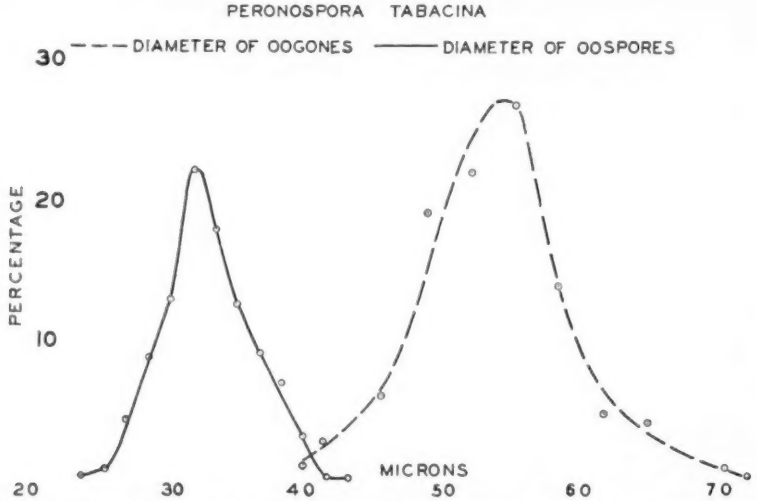

FIG. 3. Distribution of the diameters of approximately 600 oogonia and oospores of *Peronospora tabacina.*

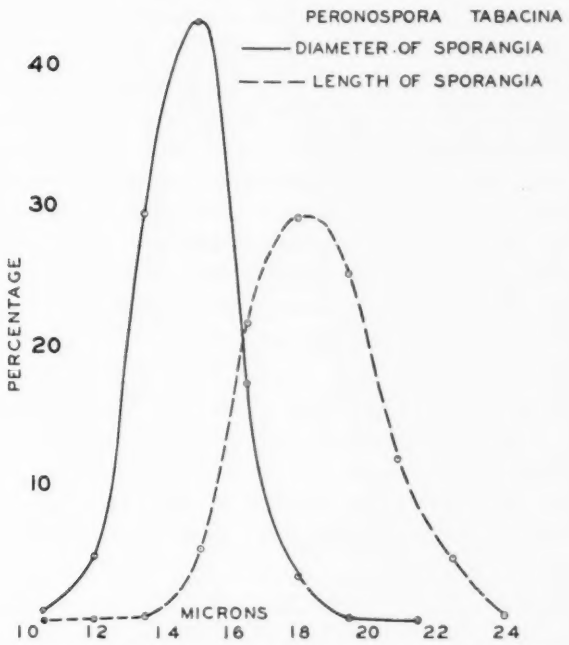

FIG. 4. Distribution of the lengths and widths of 1100 sporangia of *Peronospora tabacina*.

measurements of each structure were made in the case of oogonia and oospores. This material, from collections made in 1934, was mounted and cleared in dilute lactic acid.

From consultation of the results in figure 4, it may be seen that the range in length of sporangia varies from 10.5 to 24 μ, approximately 80 per cent being from 16 to 24 μ, with a mean length of 18.4 μ. The width of sporangia varies from 10.5 to 22 μ, approximately 85 per cent being 13 to 17 μ wide, with a mean width of 15 μ.

From figure 3 it may be observed that the oogonia range in diameter from 40 to 74 μ, approximately 75 per cent being from 47 to 57 μ, with 53 μ as a mean; and the oospores, from 24 to 43 μ, 65 per cent being 30 to 35 μ, with a mean diameter of 32 μ.

IDENTITY AND MORPHOLOGY OF PERONOSPORA SPP.[2] ON TOBACCO

Mycologists of the Old World and of North America have for years regarded the downy mildew fungus of tobacco as *Peronospora hyoscyami*

[2] The writers have examined specimens of *P. hyoscyami* de By., collected by Bakhtin, and of *P. nicotianae* Speg., both of which were loaned by S. F. Ashby, Kew Herbarium. *P. tabacina* from Australia was supplied by L. F. Mandelson, Brisbane, Australia. We acknowledge our indebtedness to both of these investigators.

de By., and that of South America as *P. nicotianae* Speg. (17). Although the sporangial stage of the former species was described in 1863 (4), its oogonial stage, collected in central Russia (Samara), was first described by Bakhtin (3) in 1926. The oogonia are 45 to 60 μ in diameter and are of the type that belong to the section Leiothecae. The oospores are yellowish brown, 30 to 44 μ in diameter, possess an irregularly folded perineum, 5 to 7 μ thick, and resemble very closely those of *P. tabacina*. The variations in measurements of sporangia are shown by the fact that those recorded by de Bary (4) are 13 to 24 × 13 to 18 μ, by Bakhtin, (4) 20 to 27 × 14 to 20 μ, and those communicated by Ashby[3] are 21 to 28 × 17 to 21 μ (mean 25 × 19 μ). The measurements by Gäumann (7, p. 320–322) range from 17 to 32 × 12 to 26 μ (mostly 21 to 27 × 16 to 22 μ) and those by Berlese, as quoted by Gäumann (7, p. 320–322) 15 to 24 × 13 to 18 (mostly 23.5 × 17 μ). These dimensions indicate that *P. hyoscyami* is larger than *P. tabacina*.

Peronospora nicotianae has oogonia of the type that belong in the section Calothecae, and it is, therefore, plainly specifically distinct from *P. hyoscyami*.

In 1933 Adam (1) made a comparative study of *Peronospora hyoscyami*, *P. nicotianae*, and the organism causing downy mildew of tobacco in Australia, and decided that the latter, although quite similar to *P. hyoscyami*, should be given a new name, *P. tabacina*. An important factor upon which the separation was based was the inability of the fungus from tobacco to parasitize *Hyoscyamus niger*. When this report of Adam appeared, our previous report (19) was in press. In it we pointed out that the downy mildew fungus of tobacco in the southeastern United States does not parasitize *H. niger* and, therefore, is not identical with P. *hyoscyami*. We concluded that it is either identical with *P. nicotianae* or else is an undescribed species. In view of the fact that type specimens of *P. nicotianae* were not available to us at that time, we preferred to regard it as *P. nicotianae*.

Recently, Clayton and Stevenson (5) determined, on morphologic grounds, that the tobacco downy mildew fungus of the United States resembles *Peronospora tabacina* and recommended that this name for it be used. Comparative morphological data, compiled from the accounts of all who have been concerned with studies on the two mildews, *P. nicotianae*, and *P. tabacina*, are assembled in table 2. These data show the present status of this matter.

Any one who examines *Peronospora nicotianae* and *P. tabacina* would most certainly decide that they are specifically distinct, because the

[3] From correspondence.

TABLE 2.—*Comparative measurements of two downy mildew fungi on tobacco*

Authority	Organism	Sporangia			Oogonia		Oospores	
		Length	Width	Mean	Range	Mean	Range	Mean
Spegazzini (17)	Peronospora nicotianae	18–20	9–11		80–100		50–80	
Ashby[a]	"	18–28	14–19	22.45 × 16.65(a)	46–85	60.4–62.3(b)	37–70.5	48.1–49.8(b)
Clayton and Stevenson (5)	"			17.44 × 16.72	62–78			
Adam (1)	Peronospora tabacina	16–29	13–19	22 × 17	35–60	46		30
Angell and Hill (2)	"	19–35	14–21	28 × 17(c)			28–50	
Wolf *et al.* (19)	"	15–28	12–18		60–85		45–75	
Wolf *et al.*	"	10.5–24	10.5–21	18.4 × 15	40–74	53	24–43	32
Clayton and Stevenson (5)	"			16.46 × 12.45			32–40	39.49
Ashby[b]	"						24–40	33.2
Ashby[c]	"						29–53	39.7

[a] Ashby, S. F., from correspondence. (a) average of 30 measurements; (b) average of 50 measurements; (c) average of 100 measurements.

[b] Measurements of 60 oospores from material from Australia supplied by Adam.

[c] Measurements of 50 oospores from material from North Carolina supplied by the writers.

oospores of the former are considerably larger, as shown by the measurements in table 2. The areolate oospore wall of the former and the smooth one of the latter is another striking difference. The lack of accord in measurements of *P. tabacina* shows that this organism is quite variable in size. The morphologic differences between *P. hyoscyami* and *P. tabacina,* however, are not very great, as previously indicated. Some might prefer to regard the tobacco pathogen as a variety of *P. hyoscyami,* since it is unable to produce disease in Hyoscyamus. Rather than assign a varietal name to the tobacco downy-mildew organism, it seems preferable, as Adam (1) has done, to assign to it a name of specific rank.

OBSERVATIONS BEARING ON CONTROL

Downy mildew of tobacco has been present in North Carolina for 5 years. It has been more destructive in some seasons than in others, but there is no evidence that it is becoming less aggressive or that it may be expected to disappear in the immediate future. On the contrary, all of the evidence in hand indicates that it will remain here and will ravage tobacco seed beds year after year. During the past spring the disease appeared on large plants in eastern North Carolina and South Carolina. This indicates that this form of the disease, reported in Georgia in 1931, and prevalent in Australia every year, may become a menace in the flue-cured areas.

Growers generally have less concern about its presence in seed beds than when the disease first appeared. They expect the disease to decimate the stands of seedlings and, consequently, they sow seed beds whose area is several times as large as would be necessary if downy mildew were absent. This increases the cost of producing tobacco for each grower. In addition, the disease makes it impossible to take advantage of propitious seasons for transplanting, and is conducive to uneven stands. Tobacco, planted either too early or too late, does not yield best quality. Both factors cause loss in revenue.

If new bed sites were universally employed each year or if there were means of adequately sterilizing the old ones, it seems highly probable that downy mildew might be entirely eradicated from the flue-cured areas. Our investigations show conclusively that the employment of sites occupied by seed beds during the previous year, constitutes the most important source of primary infections. Whether growers avoid or use old bed sites, however, is a matter of personal choice. Palliative rather than preventive measures, therefore, are most likely to be adopted by them.

There is a relationship between the age of tobacco seedlings and their chances of surviving an attack of downy mildew. The mortality in a

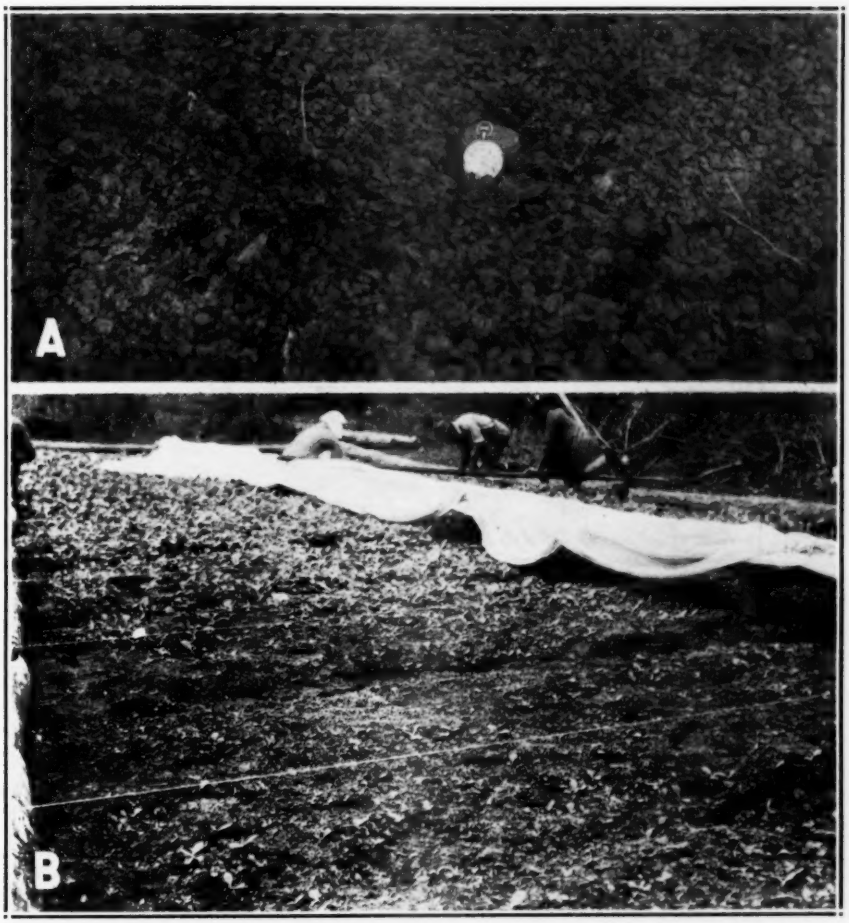

FIG. 5. A. The bare area to the left and slightly in front of the watch marks the locus of a primary infection. The seedlings were very small when photographed. B. Primarily infected seed bed near Nichols, S. C. Photographed March 28, 1935. Infection was first noted 10 days earlier. The downy mildew organism had destroyed nearly all of the seedlings in the bare area in the foreground.

primarily infected bed is always high. Such a condition is typified in figure 5. If the seedlings remain disease-free until large enough to transplant (Fig. 6), there is much less likelihood of serious losses; especially, if proper care and management of the seed beds have been provided.

Several thousand seed beds have come under our observation during the past 4 years. Evidence from these observations indicates that the location of the seed beds is an important factor in reducing the ravages of downy

FIG. 6. A. Seed bed sown on a new site near Durham, N. C. Photographed April 29, 1935, the day after infection was first evident on seedlings in the darkened area near the corner in the left foreground. B. Close view of a portion of the bed shown in A. Photographed April 29, 1935. The seedlings were sufficiently large for transplanting. Compare the size of seedlings when primary outbreak occurred (Fig. 5A) with those that were secondarily infected.

mildew. Beds should be so located as to expose the plants to the sunshine all day. It has previously been stated (19) that more severe injury from downy mildews occurs in beds shaded all or a part of the day than in those distant from the woods. The beneficial effect of sunlight cannot be overemphasized, since seedlings in heavily shaded beds may be completely

destroyed (Fig. 8, B), whereas beds located in a clearing several rods away, although infected, may produce a satisfactory crop of seedlings.

It is common knowledge that seedlings grow more slowly under thin seed-bed covers than under thick ones, and that those under the latter type are more tender and, therefore, are subject to more serious damage from downy mildew. Observational evidence indicates that the seed-bed covers should be removed about 3 weeks instead of a few days only, prior to the time for transplanting, for, by thus exposing the seedlings to direct sunlight they become hardened. This is an excellent procedure to use when repeated applications of nitrate of soda are made. The hardening effect of direct sunlight offsets the tender condition that normally results from the application of nitrate of soda. When growth of the seedlings is retarded by exposure to sunlight the disease may interfere with transplanting only slightly, or in some cases not at all.

Applications of nitrate of soda appear to hasten the recovery of downy mildew-affected seedlings (Fig. 7). This seems to result from stimulating the formation of new secondary roots near the base of the stem. A single application of sodium nitrate at time of seeding, or after the disease has appeared, is not so effective as several light applications made at intervals. The first application should be made in advance of an outbreak.

In the case of beds on old sites, primary outbreaks may be expected to occur at the time of the flowering of dogwood. All such beds should be given a light application of nitrate of soda approximately a week prior to this time, followed by another application as soon as the disease appears.

A few beds sown on new sites, and beds on old sites not evidencing primary infections, may be expected to develop secondary infections within 2 weeks after the appearance of primary foci in that locality. The disease will be general within another week. The producer, therefore, should make a light application of nitrate of soda on all beds, not evidencing primary infection, one week after the flowering of dogwood, followed by another application immediately after the disease is discovered in the seed bed. This procedure has proved of such value that numbers of observant growers have come to regard "soda (nitrate of soda) and sunshine" as the only means of reducing the damage from downy mildew and of hastening recovery from the disease.

Use has been made of various sprays and dusts as protectants. The difficulty involved in keeping the entire leaf surface adequately covered with fungicides indicates that there is little basis for hope that control will result from the use of such substances. Our limited experience shows that some of them afforded some measure of protection but that they are of little practical value. Those fungicides that produce any appreciable

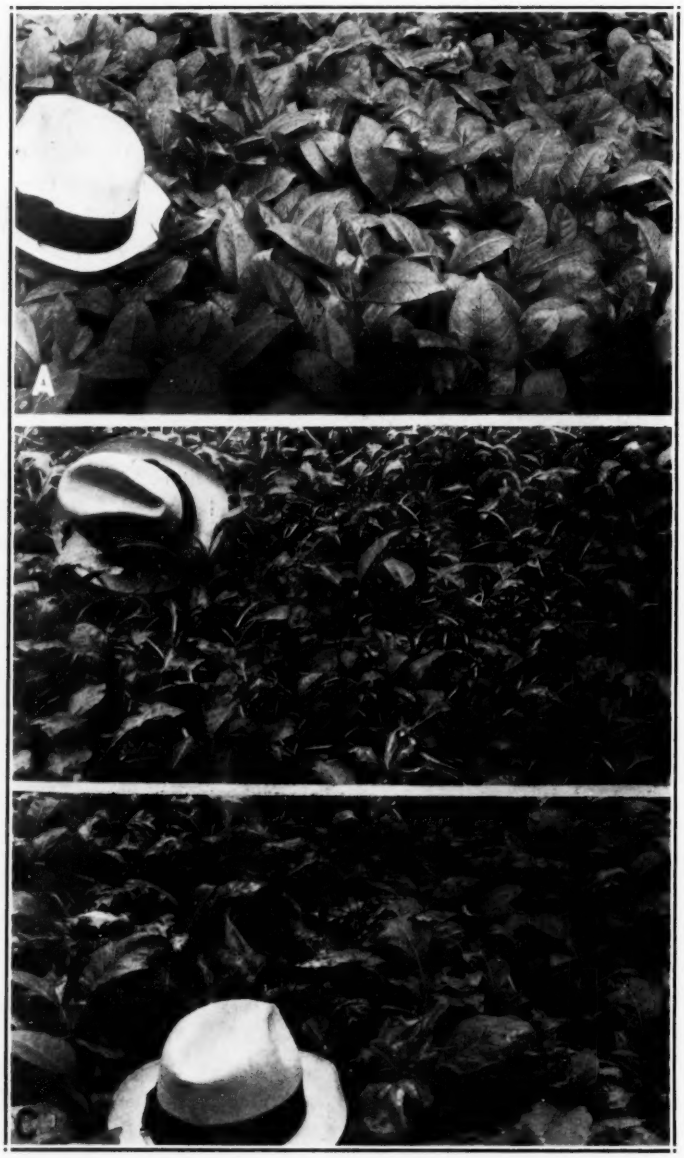

FIG. 7. Photographs taken on the same day of portions of three seed beds situated near one another. A. Nitrate of soda was applied several days prior to the outbreak of downy mildew and a second application was made while the seedlings were still severely involved. At the time photograph was taken the seedlings had recovered sufficiently to be transplanted. B. and C. No nitrate of soda applied. B. Shaded by trees during a portion of the day; the seedlings that survived had begun to recover. C. Shaded during the greater part of the day; the seedlings were severely collapsed and none were used for transplanting.

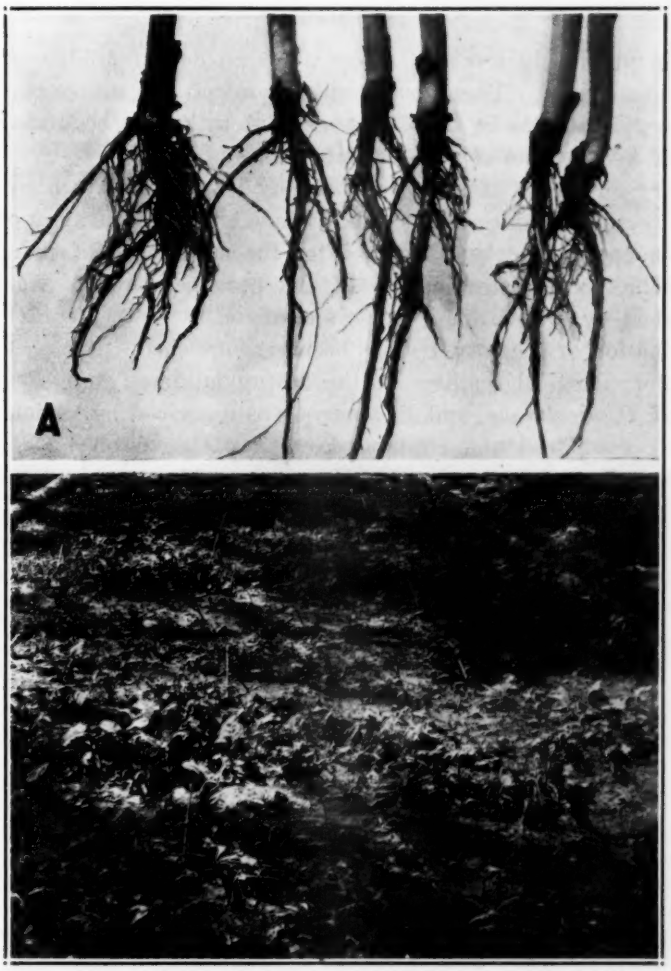

FIG. 8. A. Root systems of six tobacco seedlings. The one at the left was normal. The crowns of the remainder were discolored and they lacked secondary roots. This condition had resulted from applications of salicylanilides and naphthols as sprays. B. Tobacco seed bed closely surrounded by trees in which practically all of the seedlings were killed by the downy mildew fungus. None of the seedlings were removed for transplanting. Photographed at the close of the season for transplanting.

protection against downy mildew cause the seedlings to be stunted. Some fungicides, such as the copper salts, naphthols, and salicylanilides, interfere with the formation of secondary roots at the base of the stem (Fig. 8, A), an effect that appears to result from accumulation of the fungicide around the stems at the surface of the soil.

SUMMARY

Certain previously reported observations on downy mildew of tobacco have been confirmed. These deal with oospores as the source of inoculum for primary infections in the Carolinas, and with seed beds sown on the sites of old beds as foci of primary infection.

Primary outbreaks of the disease in any locality always occur in seed beds that occupy the sites of old beds. Secondary outbreaks are of general prevalence approximately 3 weeks after the occurrence of primary outbreaks. The general distribution of the pathogen within a locality is accomplished by means of air-borne sporangia.

Germination of the oospores has been accomplished.

The morphological features of the downy mildew fungi, *Peronospora hyoscyami*, *P. nicotianae*, and *P. tabacina*, as described by various investigators, are compared and contrasted. The pathogen on tobacco in the southeastern United States is regarded as the latter species.

It is recommended that seed beds should never be placed on old sites. This is of primary importance as a preventive measure. The most important palliative measures include the greater exposure of the seedlings to direct sunlight than is now generally employed, and the judicious administration of several applications of nitrate of soda.

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SEPARATION AND ANALYSIS OF VIRUS STRAINS BY MEANS OF PRECIPITIN TESTS[1]

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(Accepted for publication April 6, 1936)

After a rabbit is immunized with the juice of a plant containing tobacco-mosaic virus, the blood serum contains antibodies of two categories, effective, respectively, against proteins of the healthy host plant and against the virus antigen. If such a serum is treated with an adequate amount of healthy-plant juice, the antibodies against the healthy-plant proteins are removed by precipitation leaving in the supernatant fluid antibodies specific for the tobacco-mosaic disease. Such a serum is inactive toward comparable extracts containing other viruses, such as those of cucumber mosaic, various potato mosaics, and tobacco ring spot.

It is known from the work of Jensen (10), McKinney (11), and others that many strains of tobacco-mosaic virus may be derived experimentally. Similar strains also appear in nature. They are very similar in most of their properties but may be distinguished by the symptoms produced in certain hosts. Similarly, Price has shown that cucumber-mosaic virus includes distinguishable strains (12), and Böhme (4), and others have reported strains of latent potato-mosaic virus and potato-vein-banding virus. A number of investigators have attempted to use serological methods for distinguishing the strains of a given virus, but it has been found by all that serum prepared against one strain precipitates in the presence of other strains of the same virus.

In 1934 Birkeland (3) found no significant serological difference between the viruses of spot necrosis, attenuated spot necrosis, and potato ring spot, all of which are strains of the latent-mosaic virus of potato. Gratia and Manil (8), Spooner and Bawden (13), and Bawden (1) were also unable to distinguish strains of the latent potato-mosaic virus by the precipitin reaction. Beale observed that serum prepared against any one of 3 very distinct strains of tobacco-mosaic virus reacted equally well with all 3 strains employed. The writer likewise (6, 7) was unable to differentiate 20 strains of tobacco-mosaic virus from one another. Several strains of latent potato-mosaic virus were also indistinguishable from one another by means of the precipitin and complement-fixation tests. The neutralization test with the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic

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also failed in each case to differentiate strains of the same virus (5). Thus, it was evident that the strains could not be separated serologically without some further modification of the techniques.

In all of the work reported in the literature, sera prepared against one strain were tested against a number of strains in an attempt to detect quantitative differences in the strength of the reactions. It was thought, for example, that serum of an animal immunized with ordinary green tobacco-mosaic virus might precipitate juice from this strain more strongly than it would precipitate juice of a masked strain of the same virus, and vice versa. A source of error in such experiments lies in the fact that the amount of virus antigen in the various extracts may differ greatly, and the amount of antibody in two sera also may differ. Hence, quantitative differences in reactions might not be significant. In the writer's precipitin tests mentioned above, attempts were made to control this source of error by adjusting all sera to the same antibody content and by adjusting all extracts to the same virus content as determined by infection tests. But, even after taking these precautions, significant differences in the strains were not obtained. It was still thought, however, that a modified precipitin technique might reveal differences between strains of the same virus. The present paper reports the differentiation of certain strains of tobacco-mosaic virus and of certain strains of latent potato-mosaic virus by means of an absorption technique, such as was suggested by Beale in 1934 (2). The experiments herein reported were carried out with 10 strains of tobacco-mosaic virus and 3 strains of latent potato-mosaic virus. The 13 strains may be briefly characterized as follows:

1. *Tobacco mosaic* (Johnson, tobacco virus 1). Ordinary green mottling type. Caused systemic mottling in *Nicotiana sylvestris* Spegaz. and Comes.

2. *Tomato aucuba mosaic.* Yellow mottling type. Caused local necrotic spots, but no systemic infection in *N. sylvestris.*

3. *Masked tobacco mosaic* (Holmes) (9). Was systemic, but symptomless in *N. sylvestris.*

4–10. *Seven yellow tobacco-mosaic strains* isolated from field-type tobacco mosaic by Jensen (10) and designated here as J-102, J-108, J-201, J-202, J-302, J-303, and J-306. All caused yellow mottling in Turkish tobacco, and were distinguishable from one another in this host.

11. *Potato mottle.* A strain of latent potato mosaic that caused more or less ring-spot necrosis in tobacco, severe systemic necrosis in pepper, and moderate yellowish mottling without necrosis in *Datura tatula* L.

12. *Potato ring spot.* A strain of latent potato mosaic that caused severe ring-spot necrosis in tobacco, severe systemic necrosis in pepper, and severe mottling and systemic necrosis in *D. tatula.*

13. *Masked potato mottle.* A strain of latent potato mosaic that was isolated by the writer from healthy appearing potatoes. It caused systemic infection but no symptoms in tobacco and *D. tatula.* It caused a severe systemic necrosis in pepper.

These 13 viruses were propagated in Turkish tobacco. The tobacco juices were expressed, frozen, melted, centrifuged, and then used for inoculation of animals and for absorption and testing. Immune sera were obtained from rabbits, each of which was inoculated intraperitoneally 5 times at 3- to 4-day intervals with 6 cc. doses of juice containing the virus of one of the following diseases: tobacco mosaic, tobacco aucuba mosaic, masked tobacco mosaic, potato ring spot, potato mottle, masked potato mottle. Except for the absorption described below, all of the processes of the serological work were carried out according to the technique previously described (7).

To samples of serum of an animal immunized from one of the tobacco-mosaic virus strains were added progressively increasing amounts of tobacco juice containing a different strain of this virus. An analogous series of mixtures was prepared for the potato-virus strains. The series of mixtures was incubated for 2 hrs. at 37° C. and then for 16–20 hrs. at 5° C. The tubes were then centrifuged, the supernatent fluids were tested for precipitating activity against the virus extract used for absorbing, and that mixture was chosen for subsequent testing that contained the least amount of absorbent and yet did not react with the absorbent. In performing the precipitin tests subsequent to absorption, it was found desirable to use the absorbed sera without further dilution, while the test extracts were diluted 1:6, since preliminary tests had shown that this dilution gave the most satisfactory results.

Serum of animals immunized from each of 3 strains of tobacco-mosaic virus was absorbed with juices containing the viruses of the 2 other strains. These 6 absorbed sera were then tested against juices containing the viruses of the 10 tobacco-mosaic strains. The same procedure was followed with the viruses of the latent-mosaic strains. Each type of experiment was repeated 5 times with essentially the same results. The outcome of the tests is indicated in the following tables.

From table 1 it is seen that, after tobacco-mosaic-immune serum is allowed to react fully with aucuba-mosaic extract, the serum is still capable of reacting with tobacco-mosaic extract (test 1). The absorption of tobacco-mosaic serum by an excess of aucuba-mosaic juice does not remove all of the antibodies against tobacco mosaic. In other words, tobacco-mosaic virus extracts contain antigenic material that is lacking in aucuba-mosaic extracts. Similarly, the tests with aucuba-mosaic serum indicate that aucuba-mosaic extracts contain antigenic material that is absent from tobacco-

TABLE 1.—*Precipitin absorption tests for differentiating strains of tobacco-mosaic virus*

Serum immune from:	Absorbed with extract of:	No. volumes absorbent required per volume of serum	Absorption precipitate	Residual precipitin reactions when tested against extract of:									
				Tobacco mosaic	Aucuba mosaic	Masked tobacco mosaic	J–102	J–108	J–201	J–202	J–302	J–303	J–306
1 Tobacco mosaic	Aucuba mosaic	3	++++[a]	++	0	++	+	0	+	+	0	0	+
2 Tobacco mosaic	Masked tobacco mosaic	2	+++	0	0	0	0	0	0	0	0	0	0
3 Aucuba mosaic	Masked tobacco mosaic	⅓	++	0	+	0	0	+	+	0	+	+	0
4 Aucuba mosaic	Tobacco mosaic	¼	++	0	+	0	0	+	+	0	+	+	0
5 Masked tobacco mosaic	Aucuba mosaic	2	++++	++++	0	++++	+	(+)	+	+	(+)	0	0
6 Masked mosaic tobacco	Tobacco mosaic	½	+++	0	0	0	0	0	0	0	0	0	0

[a] ++++ = very strong reaction.
+++ = strong reaction.
++ = moderate reaction.
+ = reaction safely positive but not strong.
(+) = reaction weak and questionable.

TABLE 2.—*Precipitin absorption tests for differentiating strains of potato latent-mosaic virus*

Serum immune from:	Absorbed with extract of:	No. volumes absorbent required per vol. of serum	Absorption precipitate	Residual precipitin reaction when tested against extract of:		
				Potato ring spot	Potato mottle	Masked potato mottle
7 Potato ring spot	Masked potato mottle	5	++[a]	(+)	0	0
8 Potato ring spot	Potato mottle	3	+++	(+)	0	(+)
9 Potato mottle	Masked potato mottle	8	++	0	+	0
10 Potato mottle	Potato ring spot	6	++	0	+	+
11 Masked potato mottle	Potato mottle	3	++	(+)	0	(+)
12 Masked potato mottle	Potato ring spot	4	++	0	(+)	(+)

[a] See table 1.

mosaic extracts (test 4). The absorption precipitates show, however, that the extracts of these two viruses also contain common antigenic material. The antigen constitution of tobacco mosaic might be expressed as xy, that of aucuba mosaic as xz, x being the common antigenic fraction, y the fraction peculiar to tobacco mosaic, and z the fraction peculiar to aucuba mosaic. Extract of the masked tobacco-mosaic strain completely absorbed tobacco-mosaic serum and serum for the masked strain was absorbed completely by tobacco-mosaic extract, *i.e*, the masked stran has the same antigenic constitution as the green-mottling tobacco mosaic (xy), insofar as can be determined from these tests. At times a slight serological difference was seen between tobacco mosaic and masked tobacco mosaic, but this difference was neither strong nor constant. The tests indicate that the masked tobacco-mosaic strain is serologically very similar to the green-mottling tobacco mosaic from which it was derived, while aucuba mosaic differs from both in lacking antigens that are present in the other strains and in possessing antigens absent from the other strains.

It was felt desirable, once the antigenic differences between tobacco mosaic and aucuba mosaic were observed, to determine whether the yellow tobacco-mosaic strains, isolated experimentally from green tobacco mosiac

by Jensen (10), show any serological relationship to the naturally occurring yellow strain, aucuba mosaic. Table 1 gives the results when 7 of these experimentally-isolated strains were tested against the sera that differentiated tobacco mosaic and aucuba mosaic. Inspection of the table shows that 4 of the strains (strains J–108, J–201, J–302, J–303) contain the antigenic fraction peculiar to aucuba mosaic (z), and that one of them (J–201) contains in addition the antigenic fraction peculiar to tobacco mosaic and masked tobacco mosaic (y). The remaining strains (J–102, J–202, J–306) show the antigenic fraction peculiar to tobacco mosaic (y), but lack that peculiar to aucuba mosaic.

The tests with potato-mottle serum reveal a somewhat different relationship among the latent-mosaic virus strains. Mottle contains antigenic material that is not present in potato ring spot, but is present in masked potato mottle (test 10). Mottle also contains antigenic material that is absent from both ring spot and the masked virus (test 9). The 2 tests indicate that the 3 latent-mosaic strains under consideration are all serologically different. It is rather difficult to determine with definiteness the interrelations of potato ring spot and masked potato mottle, as the absorption reactions with these viruses are weak and difficult to read. However, a provisional antigenic analysis for these 3 strains based on reactions reported in table 2 suggests the following formulae: potato mottle, abc; masked potato mottle, abd; potato ring spot, ade.

In the analysis of the experiments, no mention has been made of the antigens present in diseased tobacco plants, but also present in healthy plants. This antigenic fraction, which may be designated as "p," is present in all 13 types of extracts and has been removed in all cases by absorption. The complete serological formulae, so far as present understanding permits their resolution, is then:

Tobacco mosaic:	pxy	Potato mottle:	pabc
Aucuba mosaic:	pxz	Masked potato mottle:	pabd
Masked tobacco mosaic:	pxy	Potato ring spot:	pade
J–102:	pxy		
J–108:	pxz		
J–201:	pxyz		
J–202:	pxy		
J–302:	pxz		
J–303:	pxz		
J–306:	pxy		

Previous precipitin, complement-fixation, and neutralization experiments have shown that tobacco mosaic, masked tobacco mosaic, and tobacco aucuba mosaic are all caused by strains of the same virus, as contrasted with

such distinct viruses as cucumber mosaic, tobacco ring spot, etc. By the use of absorption tests, tobacco mosaic and masked tobacco mosaic are found to be very similar serologically, while aucuba mosaic differs significantly from both. One might think that aucuba mosaic differs serologically from ordinary tobacco mosaic because aucuba mosaic is a natural variant, which has existed in nature apart from tobacco mosaic for some time. Yet the tests show that some of Jensen's yellow strains possess the antigenic peculiarities that distinguish aucuba mosaic from tobacco mosaic. The serological evidence indicates that aucuba mosaic is as closely related to tobacco mosaic as are a number of the strains isolated experimentally from tobacco mosaic by Jensen.

From the evidence reported above, it is concluded that the absorption technique used permits the differentiation of strains of the same virus type. By using such a technique it has been shown that serological differences exist among certain strains of tobacco-mosaic virus. Strains of latent potato-mosaic virus were also separable from one another serologically. It was found that the precipitin absorption technique described, not only serves to distinguish virus strains, but also gives some index of the constitution of the different viruses.

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PATHOGENICITY TESTS WITH BOTRYTIS SPP. WHEN INOCULATED INTO APPLES[1]

OTTO F. SCHNELLHARDT[2] AND F. D. HEALD[3]

(Accepted for publication October 21, 1935)

INTRODUCTION

Oudemans (7, v. 3: 495) listed 3 species of Botrytis as occurring on apple fruit. The first of these *Botrytis ramosa* Pers., is imperfectly described in Saccardo, but it is probably synonymous with *B. cinerea*. The other 2 species, *B. vera* Tr. and *B. vulgaris* Fr. are considered by Lindau (5, p. 284–285) to be synonyms for *B. cinerea* Pers. A new species, *Botrytis mali* Ruehle, (9) has been isolated from apples in Washington. According to Whetzel,[4] the binomial *Botrytis cinerea* has no sound taxonomic significance, and, until the taxonomy of this fungus can be established, the writers prefer using "*Botrytis* spp. of the cinerea type." From the evidence obtained in the pathogenicity tests and the isolations from decayed fruit in Washington it appears that *Botrytis* spp. of the cinerea type are the only species of frequent occurrence on apples in storage in this state.

Previous work (10) indicates that gray mold may become destructive as a rot of stored apples in Washington wherever there is considerable rain during the harvesting period. In certain lots of apples examined in the Wenatchee district in 1933–34, gray mold caused a greater percentage of decay than blue mold. Inoculation experiments have shown the gray mold organism to advance more rapidly on apples in cold storage than blue mold (Fig. 1). The spot-rot symptom, evident in fig. 1, A, was due to a temperature relationship, as described by Heald and Sprague, (2) and developed after the apples were removed from cold storage.

Bach[5] and Ruehle[6] found different forms of Botrytis of the cinerea type exhibiting little difference in pathogenicity. Huber[7] designated two strains

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[3] The writers acknowledge their indebtedness to the workers listed in table 1 who contributed isolations or diseased specimens for inoculation work.

[4] From letter dated November 2, 1934.

[5] Bach, W. J. The rots of apples in Eastern Washington. 45 p. 1922. (Unpub. thesis. State College of Washington Library.)

[6] Ruehle, G. D. Fungi which cause decay of apples in cold storage. 163 p. 1930. (Unpub. thesis. State College of Washington Library.)

[7] Huber, G. A. The fungous flora of the normal apple: I. The sources of contamination and spore load. II. Fungi present and their relation to decay. 103 p. 1931. (Unpub. thesis. State College of Washington Library.)

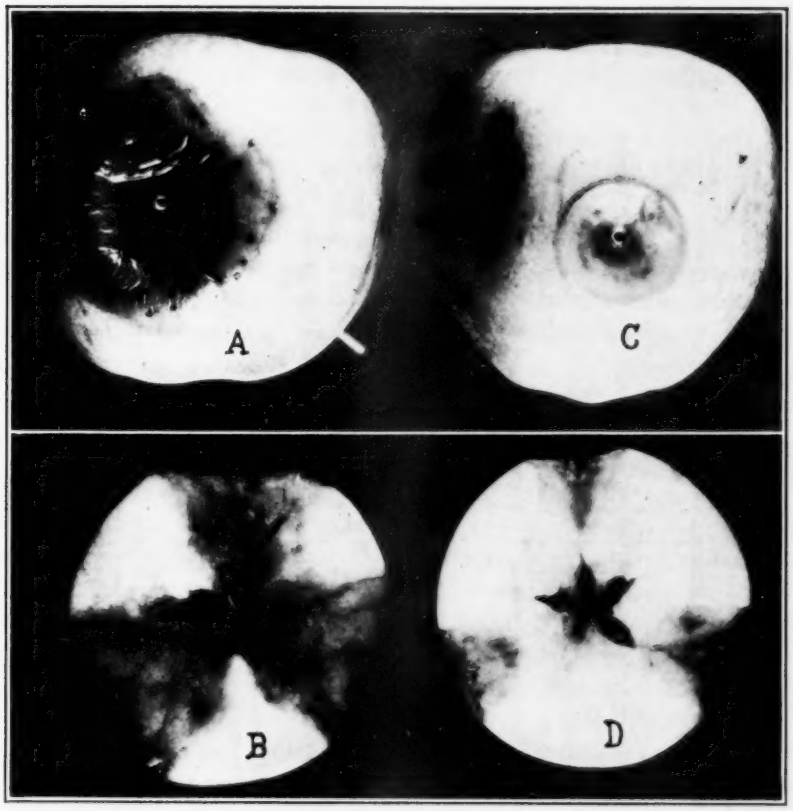

FIG. 1. Gray-mold and blue-mold lesions on Rome Beauty apples. Fruits artificially inoculated and held at 0° C. for 36 days. A and B. Gray mold (*Botrytis cinerea*). C and D. Blue mold (*Penicillium expansum*).

of the *Botrytis cinerea* type as No. 1 and No. 2 with the former producing slightly more rapid decay than the latter. Ruehle[8] found that *Botrytis allii,* isolated from an onion bulb affected with neck rot, was capable of producing a fairly rapid decay of apples when these were punctured and inoculated with spores. The onion Botrytis was not so virulent on apples as either *Botrytis cinerea* or *Botrytis mali,* and produced lesions much more slowly at the various temperatures.

MATERIALS AND METHODS

For this study various isolations of *Botrytis* spp., mostly of the cinerea type, as shown in table 1, were secured and inoculated into apples and the rate of decay recorded.

[8] *Loc. cit.*

TABLE 1.—*List of the Botrytis isolations used for inoculations into apples.*

No.	Species	Host	Locality	Worker
B– 1	B. cinerea type	Apple fruit	Pullman, Wn.	George D. Ruehle
B– 2	"	"	Massachusetts	H. H. Whetzel
B– 3	"	Pea pods	Montesano, Wn.	Self
B– 4	"	Dogwood twigs	Seattle, Wn.	G. A. Huber
B– 5	"	Blueberry twigs	Puyallup, Wn.	" "
B– 6	"	Geranium shoots	Spokane, Wn.	Self
B– 7	"	Gloxinia shoots	Spokane, Wn.	"
B– 8	"	Pear fruits	Yakima, Wn.	Harley English
B– 9	"	"	Yakima, Wn.	" "
B–10	"	"	Yakima, Wn.	" "
B–11[a]	"	Apple ?	England	Wm. Brown
B–12	B. cinerea[b]	Crassula perforata	England	W. B. Brierley
B–13	B. cinerea lini	Flax	The Netherlands	V. Beyma
B–14	B. trifolii	Clover	The Netherlands	"
B–15	B. fabae	Horse bean	Spain	J. R. Sardina
B–16	B. cinerea type	Feijoa	Riverside, Cal.	W. T. Horne
B–17	"	Guava	Riverside, Cal.	"
B–18	B. tulipae	Tulip	Pullman, Wn.	Earl Anderson
B–19	B. cinerea type	Apple fruit	Lavington, B. C.	G. E. Wooliams

[a] Cultures 11–15 were obtained from the Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands.
[b] Strain producing albino sclerotia.

Single-spore isolation cultures of these Botrytis species, with the exception of B–12 and B–13, were made according to Keitt's method (4) and used for inoculation work. Since no fructification was obtained in cultures B–12 and B–13, mycelium was used as inoculum.

Huber's method (3) being used, 15 prime maturity Jonathans were inoculated with each Botrytis culture and held at 3 different temperatures for the periods stated (Table 2). The inoculated apples were wrapped in sterile oiled wraps, and precautions were taken to prevent mixing the species of Botrytis and to exclude any outside contamination. Reisolations were made from the developing lesions and in each case the fungus used for inoculum was recovered and its identity verified by comparison with the original cultures.

RESULTS OF INOCULATIONS

The results of the pathogenicity tests indicate that *Botrytis* spp. of the cinerea type caused decay of apples at a fairly uniform rate, regardless of the host from which they were isolated. Recent isolations of *Botrytis* spp. seemed to show a more rapid advance of decay than those that had been cultured for some time. This is evident from the figures in table 2. The cultures B–1, B–12, B–13, B–14, and B–15 had been grown on artificial media some time before the inoculations were made. Three species of Botrytis, other than the cinerea type *i.e.*, B–14, B–15, and B–18, were only weakly parasitic. The data presented show an increased rate of decay as the temperature is raised and the storage period lengthened (Table 2).

The decay produced by the Botrytis isolation from *Crassula perforata* (Table 1) was a small, sunken lesion; the horse-bean isolation produced a larger, somewhat raised, smooth lesion, while the Gloxinia isolation showed a firmer, still larger lesion (Fig. 2, D, to F). The surface views of lesions of apples inoculated with Botrytis species of the cinerea type are shown (Fig. 2, A to C). Cross sections of inoculated apples showed that the fungi were fairly uniform in their rate of decay, except in B–12 and B–15, while in those two decay was slower, which is also shown by the surface of the lesions (Fig. 2, D, E). The advance of the fungi B–15, B–12, B–11 and B–7 are shown in cross section in fig. 3.

The isolation of Botrytis from Gloxinia always produced a light red pigment in the culture media and this characteristic was retained throughout the experiments. This cultural character of strains of *Botrytis cinerea* has been recognized also by Heald and Sprague (2), while Peyronel (8) in France, working with isolations from 150 hosts, obtained 12 different strains of Botrytis (of the *B. cinerea* type) that produced an abundant red pigment.

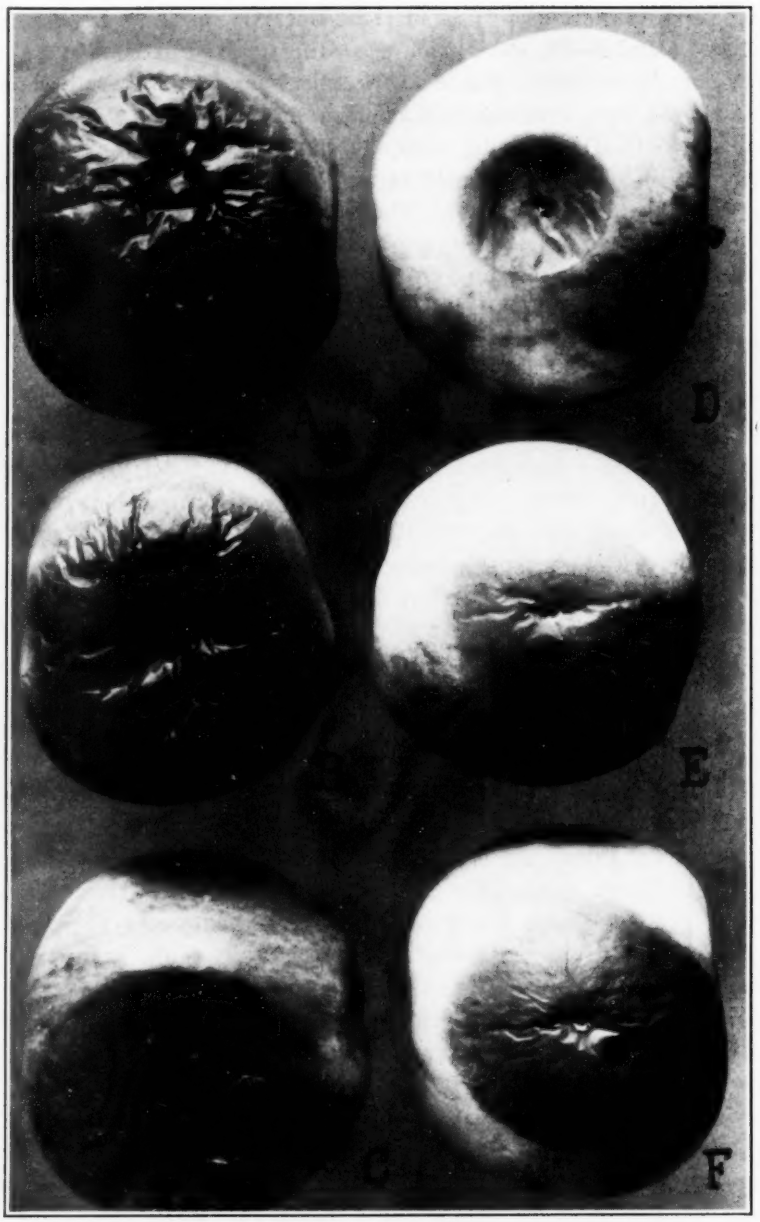

FIG. 2. Botrytis spp. inoculations showing diameter of lesions on surface of apples after 30 days at cold storage. A. B–11. B. B–2. C. B–1. D. B–12. E. B–15. F. B–7.

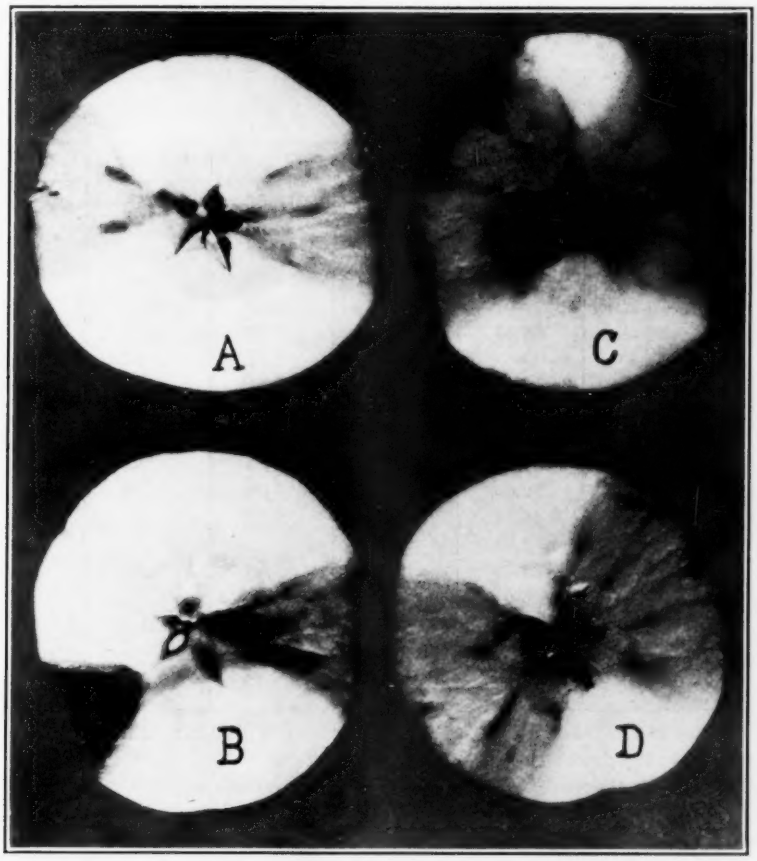

FIG. 3. Cross sections of apples inoculated with various Botrytis spp. showing advance of lesions in apple tissue. A. B–15. B. B–12. C. B–11. D. B–7.

More extensive inoculation experiments were carried on with B–1, the form previously isolated by Ruehle[9] from apples. Punctured apples of 3 varieties were dipped in a heavy suspension of spores and held in cold storage for 30- and 60-day periods (Table 3).

Two apples in the Jonathan variety and 2 in the Rome Beauty were entirely decayed at the end of the 60-day period in cold storage. These results were similar to those reported by Heald and Ruehle (1) when ripe Jonathans were one-half to three-fourths decayed at the end of a 60-day incubation period at 0° C. It seems the pathogenicity of this *Botrytis* sp. was unaffected by continued culture on artificial media.

[9] *Loc. cit.*

TABLE 2.—*Diameter in millimeters of lesions produced on sound, ripe Jonathan apples by various Botrytis spp.*

Culture Number	Room temperature (72–74° F.)		Common storage (40–40° F.)		Cold storage (32–35° F.)	
	6 days	14 days	6 days	14 days	30 days	44 days
B– 1	22.23	50.50[bb]	5.66	10.77	30.78	42.04
B– 2	28.65	58.22[cc]	8.00	17.96	44.93	64.84
B– 3	36.72	[aa]	8.87	19.47	52.60	71.29
B– 4	37.04	[aa]	8.00	20.20	53.93	73.30
B– 5	36.81	[aa]	.....	.....	47.63	65.72
B– 6	26.28	64.86	6.60	17.78	50.00	73.65
B– 7	34.80	[aa]	7.28	12.21	39.53	51.34
B– 8	28.94	[aa]	9.07	18.61	47.70	65.12
B– 9	24.14	71.16	8.08	17.66	47.06	63.91
B–10	36.85	[aa]	6.20	16.26	50.03	65.23
B–11	17.57	39.57	4.33	9.25	46.36	69.83
B–12[g]	19.00	30.66	5.66	7.06	17.14	27.54
B–13[g]	2.47[a]	46.50	.89[e]	2.25[f]	.....	.....
B–14	.00	3.23[d]	.....	.....	.....	.....
B–15	2.95[b]	33.35	.00	.00	19.26	26.86
B–16	25.57	66.61	.....	.....	.....	.....
B–17	14.38	56.42	.....	.....	.....	.....
B–18	3.42[c]	21.27	.....	.....	.....	.....
B–19	22.61	62.16	.....	.....	.....	.....

The superior letters indicate that, of the 30 punctures, a limited number developed decay as follows: a, 5; b, 7; c, 6; d, 2; e, 5; f, 11.

[aa] All lesions coalesced; [cc]Lesions on four apples coalesced.

[bb] Lesions on three apples coalesced; [g]Mycelium used for inoculum.

TABLE 3.—*Development of gray mold at cold storage temperatures (32–35° F.) when punctured apples were dipped in a spore suspension. Apples inoculated November 8, 1934*

Variety and number of apples	Average diameter of lesions in millimeters	
	30 days	60 days
Rome Beauty (28)	17.03	58.53
Jonathan (28)	12.68	33.65
Delicious (27)	28.25	53.62

DISCUSSION

Isolations of *Botrytis* spp. (mostly of the cinerea type) secured from various hosts, were used as inoculum on punctured, ripe Jonathan apples, and their pathogenicity was studied under room temperature, common storage, and cold-storage conditions. Isolations of the *Botrytis* spp. of the

cinerea type were all capable of causing rapid decay of apples, some rotting the entire apple after 14 days at room temperature. Even at the cold storage temperatures lesions of 50 mm. or more were produced at the end of the 30-day period. The Botrytis species from tulip, clover, and horse bean were only weakly parasitic. Two of the Botrytis species obtained from The Netherlands labeled and previously identified as the cinerea type, failed to sporulate and were also of little concern as parasites. The others, however, all caused a rapid decay of apples, some even greater than B–1, the original species isolated from apple by Ruehle. It appears that the Botrytis species of the cinerea type studied were strongly pathogenic on apple fruits, with the exception of B–12 and B–13. The strains that had been recently isolated from their hosts exhibited the greatest pathogenicity, while continued culturing on artificial media tended to decrease their virulence.

The results indicate that the presence of any of the *Botrytis* spp. (of the cinerea type) where apples are grown or packed may become a potential source of decay. Melchers (6) demonstrated that gray mold causing a blossom blight and leaf spot of geranium was the same as the one attacking head lettuce in the greenhouse. The limited data in this investigation tends to indicate that the gray mold on fruits, vegetables, and ornamental plants would be interchangeable in their pathogenicity. Where pears and apples are handled in the same packing houses, the gray mold infecting one could easily infect the other. Vegetables, such as peas with infected pods, might provide spores for dissemination to apple fruits. Ornamental plants, decayed by gray mold in the greenhouse and discarded outside, could supply sufficient inoculum to cause decay of apples that may be handled near by.

SUMMARY

1. Gray mold may become destructive as a rot of stored apples in Washington wherever there is considerable rain during the harvesting period. Gray mold advances more rapidly on apples in cold storage than blue mold.

2. Nineteen isolations of *Botrytis* spp., mostly of the cinerea type, were secured and inoculated into apples and the rate of decay recorded.

3. Species of Botrytis other than the cinerea type were only weakly parasitic and appear to be of little importance as a cause of apple decay.

4. Botrytis species of the cinerea type are strongly parasitic when inoculated into apples.

5. Recently isolated species seem to exhibit greater pathogenicity than those having been cultured on artificial media.

6. Plants affected with gray mold in the vicinity of apple orchards and packing houses are a potential source of inoculum that may result in decay of stored fruit.

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PHYTOPATHOLOGICAL NOTES

An Abnormal Graft Reaction in Potato Resulting from a Virus Infection of a Scion on a Resistant Stock.—In connection with studies of resistance of potato to viroses, it was found that Green Mountain scions infected with latent mosaic (X-virus) developed aerial tubers, rolling of the leaves, and marked stunting when grafted on healthy U. S. D. A. seedling number 41956 (Fig. 1, A), which is known to be resistant to latent mosaic.[1]. Green Mountain seedlings, without latent mosaic, grafted on healthy 41956 (Fig. 1, B) did not develop the above abnormalities, but Green Mountain seedlings infected with latent mosaic (Fig. 1, C) reacted like the Green Mountain. This reaction has been obtained where the Green Mountain seedlings were inoculated after the grafts were made, as well as where they were infected with latent mosaic before the grafts were made. It has been obtained in all varieties, carrying the latent mosaic that have been tested. In all instances where there was a good aerial tuber formation, there was a lack of development and early necrosis of the underground parts of the stock.

A similar abnormal reaction has been observed in grafts of mild-mosaic-infected scions on Irish Cobbler stocks, which are known to be somewhat resistant to this disease. In these grafts, the rate of formation of the aerial tubers was slow as compared with that where the latent-mosaic-infected scions were grafted on seedling 41956. This suggests that the Irish Cobbler may not be so resistant to mild mosaic as is 41956 to the latent mosaic. Thus far, 41956 has not been systemically infected with latent mosaic so that it carried through the tubers. It, however, has developed a very slight necrotic spotting of the leaves, which, in some instances, was barely noticeable, when it was grafted on a plant having latent mosaic.

Results, thus far, indicate that the general principle of grafting infected scions on potatoes to determine their reaction to the virosis concerned may be of much value in studies on resistance to viroses.—W. P. Raleigh, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Privet and Jasmine Galls Produced by a Species of Phomopsis.—

Privet.—Two hedges of the common privet, *Ligustrum vulgare* L. growing in the District of Columbia, were found badly diseased and beginning to die in patches. Examination showed that many of the stems had nodular galls near the surface of the ground; others had them on aerial parts, also. The galls ranged from 1 to 3 cm. in diameter. The largest were low down on the stems (Fig. 1, A–C). This type of gall on privet has been reported

[1] Schultz, E. S., C. F. Clark, R. Bonde, W. P. Raleigh, and F. J. Stevenson. Resistance of potato to mosaic and other virus diseases. Phytopath. **24**: 116–132. 1934.

FIG. 1. Three potato stem grafts with healthy U. S. D. A. seedling number 41956 as stocks. In Graft A the scion is a Green Mountain infected with latent mosaic; in B the scion is a virus-free Green Mountain seedling; while in C the scion is a Green Mountain seedling infected with latent mosaic. Note the aerial tubers, rolling of the upper leaves, marked stunting, and lack of development of the underground parts in grafts A and C.

from several southern States, and has been called crown gall, as though it were produced by *Bacterium tumefaciens.*

On microscopic examination, bacteria were found in the outgrowths, and a yellow bacterium was isolated from 8 galls. *Bacterium tumefaciens* did not appear on the isolation plates. On some of the older galls that were studied small pycnidia containing Phoma-like spores were observed. At first these were considered of a secondary nature.

Dr. Gotthold Steiner, the nematologist, examined some of the diseased material and found the pathogenic strawberry nematode *Aphelenchoides fragariae* (Ritzema-Bos) Steiner present in small numbers. To eliminate the strawberry nematode as the possible pathogen, it was necessary to prove that a bacterial or fungous organism was the cause of the disease. Various colonies of the yellow bacterium from the different isolations were inoculated into healthy privet plants in hedges and in pots in the greenhouse, but no infection followed.

In studying microscopic sections of the galls, so little fungus mycelium could be detected that it seemed a fungus could not be the cause of the disease. However, a Phoma was isolated from some of the younger galls and later inoculated into *Ligustrum vulgare,* both in the greenhouse and out-of-doors. Good infections followed in about 50 per cent of the inoculations made out-of-doors and in over 60 per cent of those made in the greenhouse, where the moisture conditions could be controlled better. Infections, so far as the work has gone, have occurred only through wounding the tissue when the spores are brushed on. The galls produced by inoculations reached 3 cm. in diameter in $5\frac{1}{2}$ months (Fig. 1, D).

The fungus has been reisolated and galls produced on *Ligustrum vulgare* with the reisolated pathogen. Another privet, *Ligustrum amurense* Carr., also has been inoculated with the fungus. Galls formed very slowly on this species, and up to the present time, 4 months after inoculating, are still small—less than 1 cm. in diameter. Olive, *Olea europeae* Linn., and winter jasmine, *Jasminum nudiflorum* Lindl., two shrubs related to the privet, are also susceptible to the disease, small but definite galls having been produced by inoculations with the privet gall fungus.

Four different strains of *Bacterium tumefaciens,* an organism infectious to so many unrelated plant families, failed to infect privet, while the same cultures were infectious to other hosts in the same greenhouse.

In culture, the Phoma-like pycnidia are produced in great numbers. No perfect stage has yet been found on the plants on old hibernated galls or on excised galls that had overwintered on the soil. But from galls kept 10 months in the refrigerator around 12° C., then taken out and moistened, Phomopsis spores, including the scolecospore type exuded from the pycnidia. It is not yet known if this is a new species.

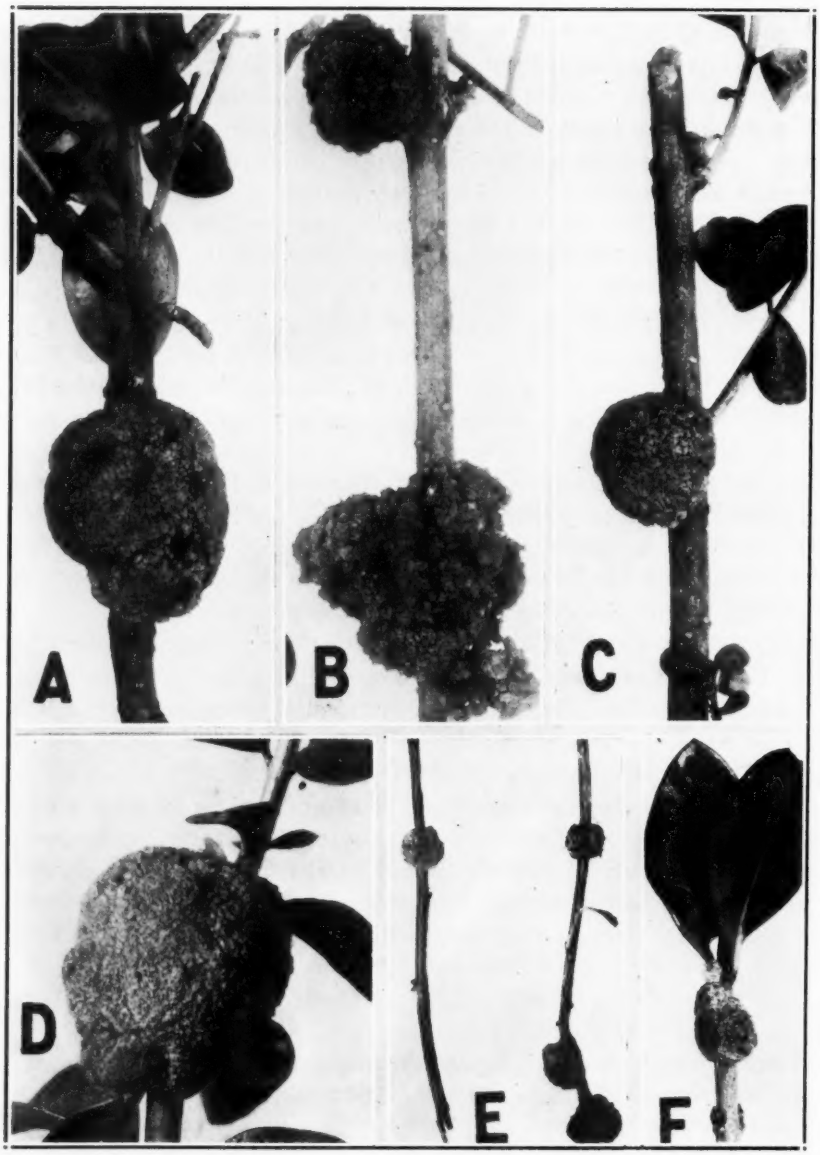

FIG. 1. A–C. Types of gall occurring on privet hedges in the District of Columbia. D. Gall produced on privet by inoculating with Phomopsis isolated from privet gall. Time 5½ months. E. Natural infection occurring on *Jasmine nudiflorum*. F. Jasmine gall produced on privet by inoculation with the Jasmine isolation. Time 1½ months. All natural size.

Jasmine.—Small galls of a finely nodular type occur in the South on *Jasminum nudiflorum* Lindl. They occur along the stems, as well as near the ground, and, when they do not weaken or kill the stem, make it unsightly (Fig. 1, E). The galls are usually smaller than those on the privet. What appears to be the same fungus found in privet galls was isolated from the jasmine and when inoculated into *J. nudiflorum* and *Ligustrum vulgare*, it produced galls readily, the jasmine galls on the privet becoming larger than those on its host plant from which it was isolated (Fig. 1, F). —NELLIE A. BROWN, Bureau of Plant Industry, Washington, D. C.

Morphological Aspects of Gymnosporangium Galls.—The morphology of the "cedar apples" on *Juniperus virginiana* L. caused by *Gymnosporangium juniperi-virginianae* Schw. has been studied during half a century, with contradictory results.

Sanford,[1] perhaps the first to study the galls, decided that they arose from leaf tissue. This opinion apparently was supported by Kern,[2] Heald,[3] Reed and Crabill,[4] and Coons.[5] Engler and Prantl[6] seem to agree with Wörnle[7] whose extensive research led him to conclude that the galls are of stem origin. Stewart[8] says that the galls arise from the axils of the leaves and evidently are transformed axillary buds.

The author has observed in extensive surveys a greater abundance of galls on awn-shape (Fig. 1, B) than on scale-like leaves (Fig. 1, A). Although the awn-shape leaves usually are considered to be the juvenile form, mature trees whose foliage is all of this type frequently are found (Fig. 1, C). The galls are abnormally abundant and appear to be attached to the stem. Careful examination, however, shows each gall as attached only to a single leaf (Fig. 1, D).

An explanation of this apparent difference in susceptibility between the two types of leaves was sought. One hundred galls taken from many trees

[1] Sanford, E. Microscopical anatomy of the common cedar apple (*Gymnosporangium macropus*). Ann. Bot. 1: 263–268. 1888.

[2] Kern, F. D. A biologic and taxonomic study of the genus Gymnosporangium. N. Y. Bot. Gard. Bull. 7: 391–494. 1909–1911.

[3] Heald, F. D. The life history of the cedar rust fungus *Gymnosporangium juniperi-virginianae* Schw. Neb. Agric. Exp. Sta. Ann. Rep. 22: 105–113. 1909.

[4] Reed, Howard S., and G. H. Crabill. The cedar rust disease of apples caused by *Gymnosporangium juniperi-virginianae* Schw. Virginia Agricultural Experiment Station. Tech. Bull. 9. 1915.

[5] Coons, G. H. Some investigations on the cedar rust fungus, *Gymnosporangium juniperi-virginianae*. Neb. Agr. Expt. Sta. Ann. Rep. 25: 217–245. 1912.

[6] Engler, A. E., and K. Prantl. Die Natürlichen Pflanzenfamilien. 1: 570. 1900.

[7] Wörnle, P. Anatomische Untersuchung der durch Gymnosporangium-Arten hervorgerufenen Missbildungen. Forstl. Natürw. Zeitsch. 3: 68–84, 129–172. 1894.

[8] Stewart, Alban. An anatomical study of Gymnosporangium galls. Amer. Jour. Bot. 2: 402–417. 1915.

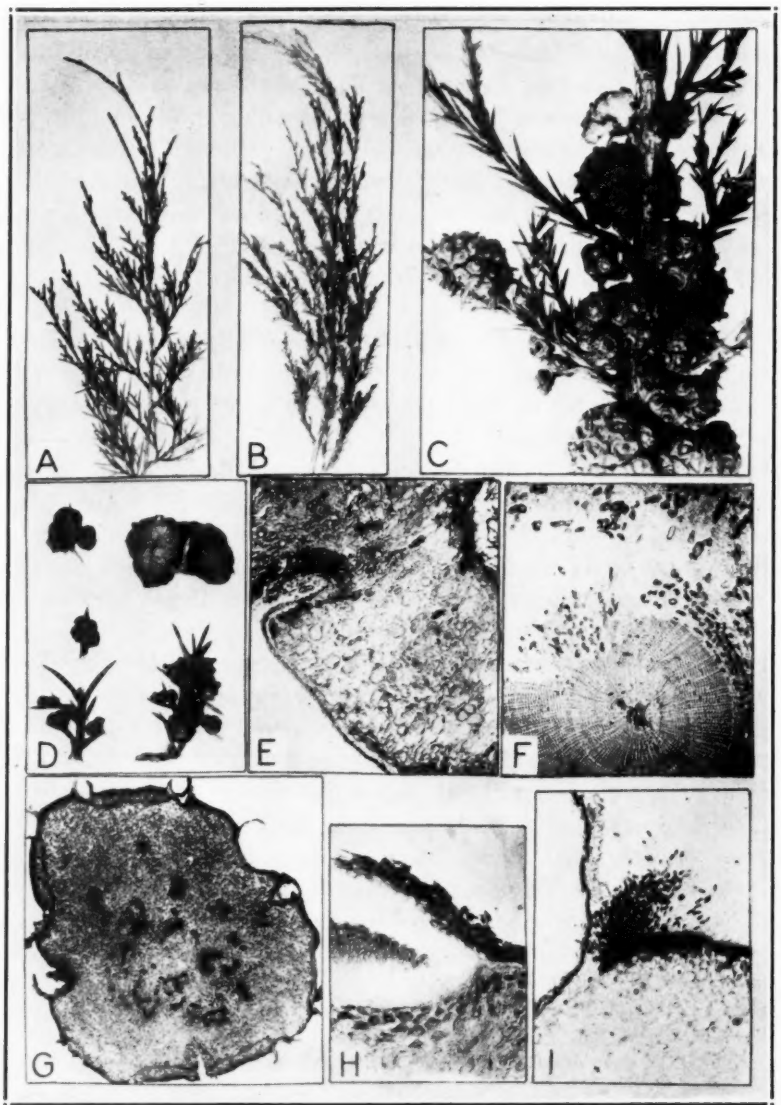

FIG. 1. A. Branch of red cedar showing the scale-like leaves or the common, mature type. B. Branch showing the awn-shape leaves, which are usually described as juvenile growth. C. Twig bearing abnormally placed galls, which are attached to the leaves of the awn-shape type. D. Galls taken from twig illustrated in "C," showing their leaf attachment. E. Photomicrograph, showing a portion of a gall which originated from an awn-type leaf. F. Photomicrograph, showing a portion of a gall attached to a stem which bore scale-like leaves. G. Thick median section of a gall showing the telial horns lifting up the cortex caps. H. A portion of a gall shortly before the absorption of water and the lifting of the cortex cap. I. Illustration of the lifted cortex cap and emergence of the spore-horn apex.

bearing the scale-like leaves and the same number from numerous trees bearing the awn-shape leaves were cut in small cubes, desilicified for 2 weeks in a 25 per cent solution of commercial hydrofluoric acid, and then prepared for sectioning by the standard celloidin process. The sections were cut 10 μ in thickness and stained with Heidenhains iron-haemotoxylin. These stained preparations indicate that the galls taken from trees with awn-shape leaves are of leaf origin (Fig. 1, E) whereas those from trees with scale-like leaves are of stem origin (Fig. 1, F).

Another phenomenon upon which opinion has been divided is the method of emergence of the gelatinous spore horns through the tough cortex of the gall. According to Stevens[9] the upper cells immediately below the surface of the cortex, where the teliospores are to form, gradually swell, become turgid, and help rupture the corky layer covering them. A technique was developed whereby clearly visible sections could be produced easily and rapidly from fresh material. Galls in various stages of development were mounted and frozen in Le Page's glue and sectioned with the freezing-microtome apparatus. The frozen glue held the gall firmly in place for sectioning, but at the same time was somewhat flexible, thus allowing thin, untorn segments to be made. Staining was not necessary to bring out the structure of the galls, as there was sufficient tannin in the fresh material. Analysis of these sections revealed circular cortex caps, lifted and pushed aside by the emerging spore horn (Fig. 1, G–I). The caps can be observed on the surface of the gall as slight pit-like depressions.—PAUL R. MILLER, Bureau of Plant Industry.

Thyrostroma compactum on Ulmus pumila.—Since the spring of 1934, the writer often has observed *Ulmus pumila* L. attacked by fungi in Illinois. In a number of instances such trees have died. The most prevalent type of fungus injury observed was the formation of cankers, with which various fungi were associated. In most instances these cankers occurred on the trunks of the trees near or at the soil line, but they have been observed also on main stems near the tops of 25-foot trees.

Among these cankers, the one shown in figure 1, A, is especially interesting. It occurred on the trunk of a 5-foot tree, just above the lowest branch and about 2 feet above the ground.

This tree had been under general observation along with many others, throughout the 1934 growing season and early spring of 1935, and had shown no striking symptoms of disease. On May 17, 1935, however, its upper portion, on which the leaves had partially unfolded, was found

[9] Stevens, Edith. Cytological features of the life history of *Gymnosporangium juniperi-virginianae*. Bot. Gaz. 89: 394–400. 1930.

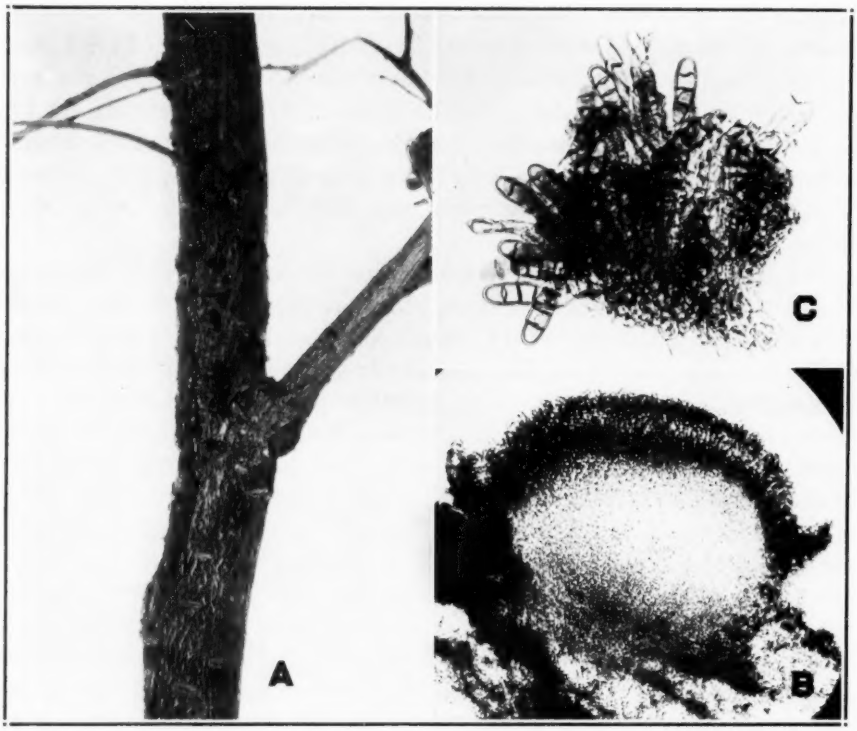

FIG. 1. *Thyrostroma compactum* on *Ulmus pumila*. A. Canker, with shrunken bark and erumpent, black tubercles. B. Tubercle in section, showing structure, relation to host tissues, and conidia on the outer surface. C. Fragment of a tubercle showing conidiophores and conidia.

wilted, the wilt involving the main stem and all lateral branches except the lowest one. Examination revealed canker.

In the cankered region the outer host tissues did not crack or slough off, but became dry, collapsed, and tightly shrunken around the stem, producing the effect of girdling either by the death of tissue or by pressure.

Some time after the appearance of the canker (3 to 4 weeks, as judged by the size of the wilted leaves) tubercles of a fungus determined as *Thyrostroma compactum* (Sacc.) v. Höhnel appeared over most of the cankered area (Fig. 1, A). These tubercles, shown in section in figure 1, B, become erumpent at maturity. They are laid down, fundamentally, in the phellogen-phelloderm region of the stem, these tissues being disintegrated wherever a tubercle develops and being replaced by a compactly interwoven hyphal mass, the base of the tubercle. The growing tubercle ruptures the overlying phellem, epidermis, and cuticle, forcing these tissues back so as to form a collar around the erumpent mature tubercle.

Hyphae within the host continue to develop at the bases of tubercles, eventually forming below each tubercle a fairly distinct, dense, brown mass that sometimes bears cavities suggestive of developing perithecial locules. This basal structure occupies the region of the cortex, endodermis, and pericycle, and, enlarging, crushes into the more or less disintegrated outer phloem, the sclerenchymatous cells of which are considerably decomposed by the vegetative fungal hyphae. Laterally from this base, a loosely interwoven subiculum-like, weft of dark hyphae develops, which replaces apparently dissolved cortex, endodermis, and pericycle.

After the tubercles have become erumpent, they appear as dark brown to black, circular, strongly convex, compact cushions protruding through the host tissue. They are 600 to 900 μ in diameter and 350 to 500 μ high.

Conidia (Fig. 1, C) are produced singly on short, tan, rod-like conidiophores over the outer face of the tubercle and accumulate as a compact mass on the top of the tubercle. They are oblong, somewhat clavate, bicellular to multicellular, muriform in part, somewhat constricted at the septa, and deep tan to brown. They range from 13.3 to 16.6 μ × 39 to 54 μ and average 14.5 μ × 46.5 μ.

While repeated attempts to germinate conidia on potato dextrose and cornmeal agar and in sterile distilled water were unsuccessful, as were also attempts to isolate the fungus from diseased sapwood, bark, and tubercles, the relationship of the fungus to the canker is obvious.

This fungus was first described by Saccardo in 1876 under the name *Coryneum compactum*,[1] but in 1882 he transferred it to Steganosporium.[2] In 1907 von Höhnel[3] transferred it to Thyrococcum, and, in 1911[4], reassigned it to his genus Thyrostroma, of which it is the type species. *Thyrostroma compactum* (Sacc.) v. Höhnel now seems to be the logically tenable binomial. It is not clear what connection, if any, exists between this and the name *Coryneum compactum* B. and Br. that appears in literature.

Since May 17, 1935, when the Thyrostroma canker was removed, the tree has been observed frequently, and, to date, there has been no further appearance of infection, indicating that excission may be a satisfactory method of control. Numerous *Ulmus pumila* L. trees were examined throughout Illinois during 1935, but were not found affected with this

[1] Saccardo, P. A. Fungi Veneti novi vel critici. Ser. V. Nuovo Gior. Bot. Ital. 8: 198. 1876.

[2] Saccardo, P. A. Fungi Veneti novi v. critici v. mycologiae Venetae. Addendi . . . Ser. XIII. Michelia 2: 542. 1882.

[3] Höhnel, F. von. Fragmente zur Mykologie. Sitzber. k. Akad. Wiss. Wien Math. Naturw. Kl. 116: 154. 1907.

[4] Höhnel, F. von. Fragmente zur Mykologie. Sitzber. k. Akad. Wiss. Wien Math. Naturw. Kl. 120: 472. 1911.

canker.—J. C. CARTER, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

A Die-back of Douglas Fir.—During the summer of 1935, specimens of a die-back of Douglas fir (*Pseudotsuga taxifolia* Brit.) were received for examination at the New Haven Office of the Division of Forest Pathology, U. S. Department of Agriculture. The leaves of the current season's growth were pale yellow with small black pycnidia at the base or scattered the entire length of the needles. Most of these pycnidia contained brown unicellular spores, but a few hyaline unicellular and brown bicellular spores were among them. Since the spores were predominantly unicellular, the fungus was identified as a species of Sphaeropsis.

Inspection of affected trees, located on an estate at Southold, Long Island, showed conspicuous infection of terminal growth on the lower branches. The young twigs had been killed back to the nodes, but no defoliation had occurred. The leaves of the previous season's growth showed no indication of disease. On the twigs, however, definitely defined cankers with small erumpent pycnidia of the fungus extended back from the nodes to about one-half the length of that year's growth. None of the Douglas firs in the planting were entirely disease-free, although some were much more heavily infected than others.

Several Austrian pines, *Pinus nigra* Arn.; a white pine, *P. strobus* L.; and a Colorado blue spruce, *Picea pungens* Engelm., near the Douglas firs, showed a similar die-back and infection by a species of Sphaeropsis. A study of material from the 4 hosts indicated that the pycnidia, spores, and cultural characteristics of the organisms isolated were very similar. Measurements of 50 brown unicellular spores from pycnidia on the needles of each of these hosts showed a range of 24–40 μ × 9–15 μ. The length of the majority of the spores fell within the following ranges: Austrian pine 30–37 μ, Douglas fir 26–31 μ, white pine 28–34 μ, spruce 26–30 μ. Spores from pycnidia on the twigs of Douglas fir corresponded closely with those from Austrian pine, 50 brown unicellular spores of the former showing a range of 27.6–38.8 μ, the majority being within the range 29–35.6 μ. This difference may be due to variation in spore maturity.

Numerous reports have been made of the occurrence of a twig and needle blight attacking various species of 2-, 3-, and 5-needle pines, including *Pinus nigra*, *P. strobus*, and *P. sylvestris* L., with which is associated a fungus identified as *Sphaeropsis ellisii* Sacc. [1,2] or as *Diplodia pinea* (Desm.) Kickx

[1] Hedgcock, G. G. Notes on the distribution of some fungi associated with diseases of conifers. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reptr. **16**: 28–42. [Mimeographed.] 1932.

[2] White, R. P. Notes on new or unusual outbreaks of diseases of ornamentals in New Jersey in 1935. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reptr. **20**: 79–81. 1936.

(*Botryodiplodia pinea* (Desm.) Petr.).[3,4] No disease of Douglas fir caused by a species of either of these genera has been reported in the United States, so far as known. The descriptions of the disease and the accompanying fungus on the pines, however, seem closely applicable to the disease and fungus of the Douglas firs. A specimen of this latter host from near St. Helena, California, showing a definite canker, with pycnidia[5] at the node of a living branch, was collected by J. S. Boyce in July, 1930. The pycnidia and spores on this canker correspond with those on the Long Island specimens, measurements of the brown unicellular spores showing a range of 26–35.6 × 9.6–14.8 μ.

A similar disease of Douglas fir has been reported from Italy by Petri[6] who suggested that it had spread to the firs from neighboring Scotch pines. In New Zealand[7] the death of Douglas fir saplings in a young exotic forest has been attributed to infection by *Diplodia pinea* following a protracted drought.

Petri stated that the susceptibility of the Douglas firs in Italy was correlated with unfavorable growing conditions, such as excessive moisture, insufficient light, and low temperatures. Although these particular conditions would not be applicable to the trees in the Long Island location, it is undoubtedly true that the situation, in a light sandy soil and outside the natural range for Douglas fir, is inimical to normal development, and that the trees may have been weakened by drought, followed by unusually severe winter conditions.—ALMA M. WATERMAN and J. ARMSTRONG MILLER, Division of Forest Pathology, Bureau of Plant Industry. In cooperation with the Osborn Botanical Laboratory, Yale University, New Haven, Conn.

Cytosporina ludibunda on American Elm.—During the summer of 1934 and, to a lesser degree, that of 1935, *Cytosporina ludibunda* Sacc. was observed to be prevalent on American elms in Illinois. Attacked trees exhibited a wilting and dying back of the diseased branchlets, followed by the formation of cankers when infection reached larger and older parts. Fruiting of the fungus occurred in the cankered areas. Successful inoculations have been made with pure cultures of the fungus on potted American elms.

[3] Schwarze, C. A. The parasitic fungi of New Jersey. New Jersey Agr. Expt. Sta. Bull. 313. 1917.

[4] Curtis, K. M. A die-back of *Pinus radiata* and *P. muricata* caused by the fungus *Botryodiplodia pinea* (Desm.) Petr. New Zealand Inst. Trans. and Proc. **55**: 52–57. 1926.

[5] Identified as a species of Sphaeropsis by G. G. Hahn, Division of Forest Pathology, Bureau of Plant Industry, U. S. D. A.

[6] Petri, L. Disseccamento dei rametti di *Pseudotsuga Douglasii* Carr. prodotto da una varietà di *Sphaeropsis ellisii* Sacc. Ann. Mycol. **11**: 278–880. 1913.

[7] New Zealand. State Forest Serv. Ann. Rept. Dir. Forestry for the year ended March 31, 1934. 17 pp. 1934. Abs. in Rev. Appl. Mycol. **14**: 65. 1935.

A series of 5 trees were inoculated between March 6 and May 14, 1935. All inoculations were made on terminal foliage and wood as the buds were opening. The leaves and wood were sterilized with 1/1000 $HgCl_2$ and rinsed in sterile water. On 3 trees, the sterilized leaves and twigs were injured by pricking with a sterile needle previous to inoculation, to provide a direct avenue of entrance for the fungus, while on 2 trees the sterilized parts were not injured. In each case, a test tube containing a growing culture of *Cytosporina ludibunda* Sacc. on slanted potato-dextrose agar was placed over the twig to be inoculated and held with a ring stand, so that the fungus colony was kept in contact with the sterilized leaves and wood. The test tube was plugged with cotton to prevent contamination.

After intervals ranging from 25 to 54 days, signs of successful inoculation became apparent and symptoms of disease continued to develop through varying periods, the first tree wilting completely in 54 days and the last after the lapse of 7 months. Reisolations were attempted, first, when death of inoculated twigs had progressed downward 8 to 10 inches from the region exposed to inoculation and, second, when the trees had wilted completely. From each tree platings on corn-meal agar were made of tissue taken at points 4, 8, and 12 inches below the place of inoculation and 4 inches above the soil line.

The uninjured trees, though having shown external signs of successful inoculation, presented no internal symptoms of infection, and the attempts to reisolate the Cytosporina from them were all unsuccessful.

The injured trees, however, gave approximately the same symptoms observed in trees naturally infected, including particularly the light brown discoloration of parts of the xylem. Attempts to reisolate the fungus from these trees were successful. From one the Cytosporina was secured 8 inches below the point of inoculation; from another, at the base and at 12 inches, but not at 4 or 8 inches, below the point of inoculation; and from the third at all 4 of the points tried.—J. C. CARTER, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

BOOK REVIEW

Wollenweber, H. W., and O. A. Reinking. *Die Verbreitung der Fusarien in der Natur.* 80 pp., illus. R. Friedländer und Sohn. Berlin. 1935.

The book consists of an introduction, 8 lists of Fusaria in cross-index form and in different groups; and a chapter, with 12 figs., on genera of fungi other than Fusarium, some members of which formerly had been included in Fusarium, but no longer can be retained there. In the introduction are briefly described the biology, the nature of former and present records of occurrence, systematic relationship with other Hypho- and Ascomycetes, the relative parasitism and saprophytism, the relative usefulness of former and present host records, and of occurrence in the air and soil, of the fungi of the genus Fusarium. The systematic details are omitted in this book because these just recently have been published in another text[1] by the same authors.

The list of Fusaria given in this book are as follows: (1) Of all names of Fusarium species and varieties, with forms, now accepted by the authors, and with corresponding Ascomycetes and references to the illustrations given in Wollenweber's *Fusaria autographice delineata,* tables 1–1200; (2) of Fusaria arranged in sections and subsections, with citations to the 28 illustrations given in this book; (3) of Fusaria occurring on plants, fungi included, on living animals, on raw materials and products of plants and animals, and also found in soil, air, and water—here under each of the Fusaria, are given the hosts, substrata, and media in or on which the fungus has been found. The 5 remaining lists are those of special groups of Fusaria—(4) on higher plants, given under generic names (about 400) of the hosts alphabetically arranged; (5) on algae, lichens, and mosses; (6) on other fungi; (7) on animals; and (8) on raw materials and products of plants and animals, also in soil, air, and water.

The subject is presented in this book apparently with remarkable thoroughness and is brought up to date. The references given here, in the list of Fusaria, to the 1200 illustrations in Wollenweber's *Fusaria autographice delineata,* and the fact that here the list of the finally accepted names is identical with that in *Die Fusarien,* make this little book very valuable to all interested in these fungi. In a way, it completes the previous works of Wollenweber and makes his *Fusaria autographice delineata,* and also his and Reinking's *Die Fusarien,* directly and more readily usable. It should be stated also that the general lists of the fungi and hosts, and of the groups of them, provide extremely convenient means to locate quickly previous records of the fungi.—C. D. Sherbakoff, University of Tennessee, Knoxville, Tenn.

[1] Wollenweber, H. W., und O. A. Reinking, Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. 355 pp., Paul Parey, Berlin, 1935.